ISJ 14: 340-351, 2017 ISSN 1824-307X

RESEARCH REPORT

Bacterial communities in gills and intestines of yesso scallop *(Patinopecten yessoensis)* and its habitat waters in Changhai (Dalian, China)

G Lu[1], F Wang[1], Z Yu[1], M Lu[1], Y Wang[1], C Liu[1], Z Xue[1], Y Wu[1], L Wang[1,2], L Song[1,2]

[1]Liaoning Key Laboratory of Marine Animal Immunology and Disease Control, Dalian Ocean University, Dalian 116023, China
[2]Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China

Accepted September 5, 2017

Abstract

Yesso scallop is a marine bivalve mollusc of economic importance in the coastline of northern China. The frequently outbreak of various diseases has heavily threatened the sustainable development of the scallop industry. The information about the bacterial communities inside and outside the body of Yesso scallop will provide insights into disease prevention, probiotic application and health aquaculture. In the present study, the diversity of bacterial communities in intestines, rectum and gills of Yesso scallop and its habitat waters were investigated. The bacterial diversity and richness in waters were higher than that in intestines, rectum and gills. The microbiota from intestines, rectum, gills of scallop and waters were clearly separated into four clusters by Non-metric multidimensional scaling and hierarchical cluster analysis, suggesting a microbiota selection of scallop at organ scale. Venn diagram suggested that the bacterial community in the body of scallop was more specialized than that in waters. High-throughput sequencing revealed that the main bacterial communities in intestine, rectum and gill were Firmicutes, Tenericute and Proteobacteria, Chlamydiae, Proteobacteria and Firmicutes, and Proteobacteria and Firmicutes, respectively. While the bacterial communities in waters mainly included Proteobacteria, Bacteroidetes and Cyanobacteria. Real-time PCR for bacterial load showed that the total bacterial abundance in waters was significantly higher than that inside the scallop body. The abundance of *Bacteroides fragilis* in rectum was significantly higher than that in intestines and gills. The results about the bacterial community inside and outside the body of Yesso scallop are helpful to better understand the relationship between the symbiotic bacteria of Yesso scallop and their habitat, and also provided critical information to develop strategies of disease prevention and probiotic application in scallop aquaculture.

Key Words: *Patinopecten yessoensis*; bacterial diversity; habitat waters; gills; intestines; high-throughput sequencing; real-time PCR

Introduction

Microbiota refers to the microbial community harbored in a specific ecosystem, and the commensal microbiota associated with a host plays a critical role in the health of the host organism (Ruby *et al*., 2004). The interaction of host-microbiota is essential to many processes of normal physiology, including immune homeostasis, metabolic activity and host development (Rawls *et al*., 2004). With the great contribution of the interaction between host and microbiota, scientists are becoming increasingly aware of the coevolution of animals and microorganisms and the relationship between host health and commensal microbiota (Xu and Gordon, 2003; Lai *et al*., 2009; Goffredi *et al*., 2014). There are more and more reports on the classification and functional diversity of the microbial community, especially in marine invertebrates whose mortality is an important global concern for marine ecosystems (Lafferty *et al*., 2004; Plowright *et al*., 2008; Ngangbam *et al*., 2015; Nielsen *et al*., 2015).

The composition of microbial communities could be strongly controlled and regulated by the external environments (Fierer and Jackson, 2006).

Corresponding author:
Linsheng Song
Liaoning Key Laboratory of Marine Animal Immunology and Disease Control
Dalian Ocean University
Dalian 116023, China
E-mail: lshsong@dlou.edu.cn

Table 1 Characteristics of sampling sites

Site	Longitude	Latitude	Temperature (°C)	Salinity (‰)	DO (mg/l)	pH
A	39°17′23″N	122°44′18″E	12.36±0.45	32.11±0.34	8.27±0.39	8.26±0.19
B	39°17′21″N	122°44′59″E	12.34±0.31	32.04±0.29	8.78±0.67	8.37±0.08
C	39°17′15″N	122°45′12″E	12.04±0.42	32.00±0.19	8.80±0.55	8.42±0.07

The microbiota in marine invertebrates has been extensively studied, and the formation of microbiota was believed to be affected by many factors, such as host habitat (Klaus *et al*., 2005), individual host characteristics (Cardenas *et al*., 2014), host health level (Lu *et al*., 2013), organ compartmentalization (Meisterhans *et al*., 2016), diet and development stages (Rungrassamee *et al*., 2013; Miyake *et al*., 2015). There is a selective association between invertebrates and the related microbes. For instance, the composition of bacterial communities in intestine and habitat environment of shrimp (*Litopenaeus vannamei*) was reported to be influenced by habitat environment (Peng *et al*., 2006; Luo *et al*., 2009). The dominant bacteria members in crab (*Eriocheir sinensis*) intestines were Tenericutes and Proteobacteria, while Actinobacteria, Proteobacteria and Bacteroidetes were dominant in its habitat waters (Zhang *et al*., 2016). The bacterial diversity in the habitat surface sediment and intestine contents also showed significant difference in sea cucumber (*Apostichopus japonicas*) (Gao *et al*., 2014). The accumulating evidences suggest that the composition of aquatic microbial communities in habitat waters has a strong influence on the symbiotic microbes of farmed aquatic animals (Peng *et al*., 2006). However, the information about the relationships between the symbiotic microbiota and the habitat of Yesso scallop is still very limited.

The host microbiota structure is always different among organs, indicating that there may be a selection at organ scale (Meisterhans *et al*., 2016). For example, the microbial compositions in coral surface mucus layer and skeleton were significantly different (Sweet *et al*., 2011). The symbiotic microbiota in crab (*Eriocheir sinensis*) was found to be body site-specific in intestine and gill (Zhang *et al*., 2016). Comparison of microbial diversity along human intestinal tract suggested that the microbial community in jejunum was different from those in ascending colon, rectum and distal ileum (Mei *et al*., 2005). In Easter oyster (*Crassostrea virginica*), microbial community in intestine also differed from that in stomach and harbored a relatively multiple assemblage of phylotypes (King *et al*., 2012). In adult yellow grouper (*Epinephelus awoara*), the autochthonous microbiota was variable in different parts of the digestive tract (Zhou *et al*., 2009). These results suggested that the variability of microbiota among organs was associated with their respective physiological roles (Meisterhans *et al*., 2016). However, the symbiotic microbiota in Yesso scallop especially in intestine is still not clear.

Yesso scallop (*Patinopecten yessoensis*) is a low-temperature bivalve, which mainly distributes in the coastline of northern Japan, northern Korean Peninsula and Far East of Russian (Hou *et al*., 2011). It has become one of the most important farmed marine molluscan species in northern China since it was introduced into China in 1980s (Wang, 1984). However, the frequent outbreaks of infectious diseases have caused massive mortalities and heavy economic losses in the past years (Li and Xue, 2005; Yuan *et al*., 2010). There are many factors contributing to the high mortality of cultured scallops such as pathogens, pollutants, elevated temperature, aquaculture practices and physiological stress (Xiao *et al*., 2005). The symbiotic microbiota and habitat environment have been recognized as the most important factors impacting on the fitness of aquatic animals (Li *et al*., 2007b; Liu *et al*., 2011;Yan *et al*., 2012; Ye *et al*., 2013). Characterizing the bacterial community in Yesso scallop and its habitat waters is an important step to learn the function of symbiotic bacterial in disease prevention and probiotic application for scallop culture.

In the present study, high-throughput sequencing analysis of 16S rDNA gene was employed to study bacterial diversity in intestine, rectum and gill of Yesso scallop and its habitat waters in January from three parallel sites in Changhai, Dalian, China. The abundance of the total bacteria and *Bacteroides fragilis* load were examined by real-time quantitative PCR. Our objectives were (1) to find the difference of bacterial community at organ scale, (2) to understand the relationship between the symbiotic bacteria of Yesso scallop and their habitat, and (3) to provide theoretical basis for the development for disease prevention and probiotic application in Yesso scallop farming.

Materials and Methods

Sample collection

Yesso scallops (*Patinopecten yessoensis*) and seawater were collected from three parallel sampling sites (Table 1) in a Yesso scallop farm in Changhai (Dalian, China) in January 2017. There were three parallel sample sites with average distance of 0.2 km. The temperature, salinity, dissolved oxygen (DO) and pH content of the sampled seawater were measured using a portable meter (HACH, USA). About two liters of surface seawater was collected per

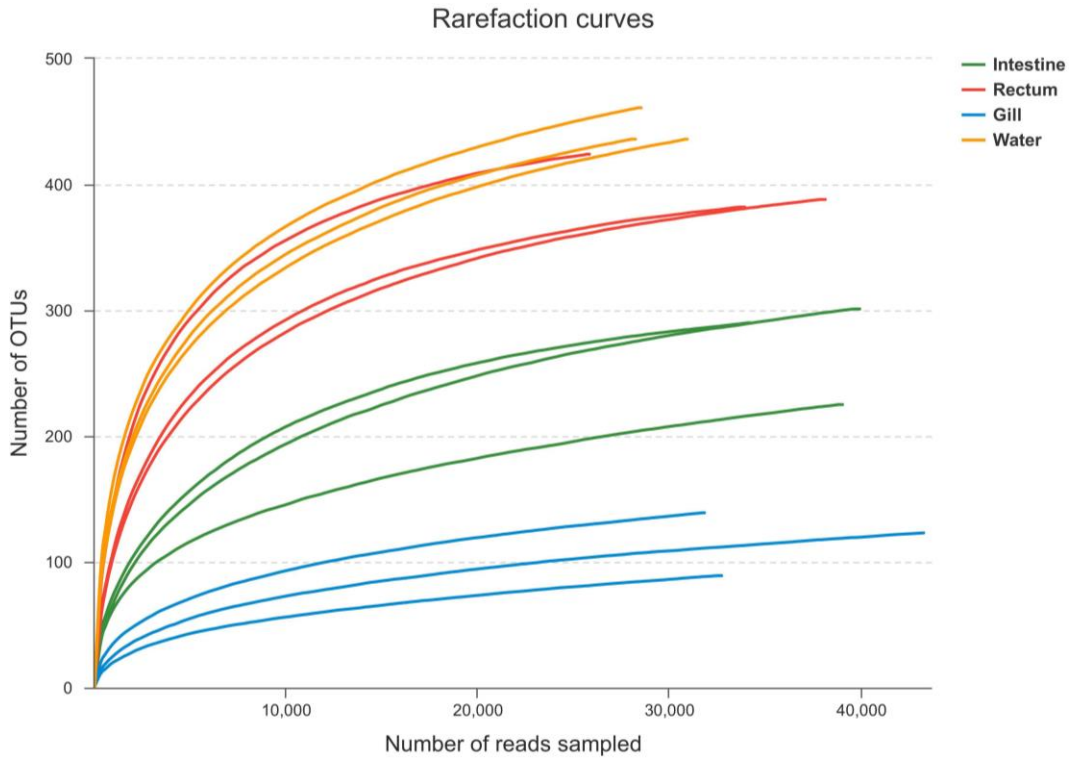

Fig. S1 Rarefaction curves of the bacterial 16S rDNA gene sequences from intestines, rectum, gills of Yesso scallop and its habitat waters. Rarefaction curves of operational taxonomic units (OTU) were clustered at 97 % sequence similarity level across different samples.

sampling site and filtered through a 0.22 μm polycarbonate membrane (Sangon, China) under sterile conditions. The membrane was transferred to sterile microfuge tubes and stored at -80 °C before bacterial genomic DNA extraction. Twelve Yesso scallops of 1-year-old with an average weight of about 10 g were collected from each parallel sampling site. The shell surface of scallops was washed with sterile seawater for three times and disinfected with 70 % ethanol to wash away debris attached on the surface. After the shells were opened, scallops were washed with sterile water for three times. Intestines (IN), rectum (RE) and gills (GI) were aseptically isolated according to the previous reports (Prosser *et al*., 1965; Fritsch *et al*., 1976), and stored at -80 °C before bacterial genomic DNA extraction.

Genomic DNA extraction and high throughput sequencing

The same tissue obtained from each site was mixed together prior to DNA extraction. Total bacterial genomic DNA was extracted from the samples according to the manufacturer's instructions of E.Z.N.A.Water DNA Kit (for seawater samples) and E.Z.N.A.Soil DNA Kit (for tissue samples) (OMEGA, USA). The quality and quantity of the genomic DNA were examined by 1 % agarose gel electrophoresis and measured by UV/Visible Spectrophotometer at 260 and 280 nm. The extracted DNA was stored at -20 °C for further sequencing and real-time PCR analysis. High throughput sequencing of V3-V4 region of 16S rDNA gene was performed by Majorbio Co., Ltd (Shanghai, China) based on the IlluminaMiseq sequencing platform.

Real-time quantitative PCR

In this study, absolute quantitative PCR was conducted to determine the number of total bacteria and 10 *B. fragilis* group species. 16S rDNA genes of bacteria and *B. fragilis* were amplified by rTaq DNA polymerase (Takara, Japan) using primers 27F/1492R (Goodfellow and Stackebrandt, 1991) and BfrF/BfrR (Liu *et al*., 2003), respectively. Standard plasmid was constructed by inserting the amplicon into pMD18-T vector (Takara, Japan) and transformed into *Escherichia coli* Trans5α (TransGen, China). After verified by sequencing, the plasmids were extracted from *E. coli* with column plasmid preps kit (Sangon, China). The concentrations of recombinant plasmids were determined by Nanodrop ND-1000 (Thermo, USA), and the copy number of standard plasmid was calculated according to the method as previously reported (Liu *et al*., 2016). Standard templates (10^3-10^9 copies of target genes) were diluted at a ratio gradient of 1:10 in DEPC water according to the copy number. The dissociation curve of real-time PCR with each diluted templates was used as quantification standards for samples. The threshold cycle (Ct) values against the denary logarithms of

the copy numbers were recorded and analyzed to set up copy number standard curve. Primers 341F/534R (Liu *et al*., 2011) and BfrF/BfrR were used to specifically estimate the total bacteria and *B. fragilis* load, respectively. Bacterial genomic DNA of different samples was adjusted to the final concentration of 10 ng/μl for real-time quantitative PCR. The Ct values were recorded and analyzed after real-time quantitative PCR assay. The abundance of the total bacteria and *B. fragilis* were calculated based on standard curve and Ct values.

Bioinformatics and statistical analyses

The raw Illuminafastq data were processed using Mothur v.1.11.0 (http://www.mothur.org/) to eliminate the low quality and redundant reads. A window with 50 bp was set to filter reads tail mass value of < 20 bp. Bases were cut at the end of the window with the average quality score of < 20. Operation taxonomic units (OTUs) were clustered with 97 % similarity by Usearch (vsesion 7.0; http://drive5.com/uparse/) against the SILVA database (release128; http://www.arb-silva.de) (Edgar, 2013). Chimeric sequence and a single sequence without duplication were removed (http://drive5.com/usearch/manual/singletons.html) (Edgar *et al*., 2011). Each 16S rRNA gene sequence without chimera was analyzed by the Ribosomal Database Project (RDP) Classifier with a 70 % confidence threshold (version 2.2 http://sourceforge.net/projects/rdp-classifier/). Sequencing depth was determined using rarefaction curve by Mothur software. The Shannon, Chao and Coverage indices were calculated for each sample using the methods of Mothur (Shannon, 1948; Chao and Lee, 1992; Edgar *et al*., 2011; Larsen *et al*., 2014). Community composition barplot and pieplot were drawn using R script. Non-metric multidimensional scaling (NMDS) and hierarchical clustering trees were constructed with weighted UniFrac distance. Venn diagram was plotted based on the OTUs in all samples from intestines, rectum, gills and waters. Real-time PCR data were analyzed by Statistical Package for Social Sciences (SPSS) 17.0 software. One-way analysis of variance (ANOVA) was used to test the significant differences among all samples and the differences were considered significantly at $p < 0.05$. The figures were pictured by Origin 8.0.

Results

General information on sampling sites and overview of 16S rDNA high-throughput sequencing analysis

Three parallel sampling sites in Yesso scallop farm in Changhai were used in this study. The temperature, salinity, dissolved oxygen and pH was 12.04 - 12.36 °C, 32.00 - 32.11 ‰, 8.27 - 8.80 g/l and 8.26 - 8.42, respectively (Table 1). High-throughput sequencing of the 16S rDNA gene amplicons was performed to determine bacterial diversity in intestines, rectum and gills of Yesso scallop as well as its habitat waters. In the rarefaction curves, the number of OTUs almost reached the plateau phase with the increasing read number at 30,000 (Fig. S1), suggesting the sufficient sampling depth in all samples. A total of 447,232 sequences and 692 distinct operational taxonomic units (OTUs) were obtained from 12 samples with an average read length of 445 bases. There were 475, 386, 188 and 553 OTUs in rectum, intestine, gill and seawater samples, respectively (Table 2). The number of bacteria genera was highest in waters (234 genus), whereas lowest in gill (67 genus) (Table 2).

Bacterial community dissimilarity among different samples

To estimate and compare the bacterial diversity among different samples, the proportion of OTUs was used to calculate community richness and diversity indices. Good's coverage index was used to estimate the percentage of total bacterial OTUs in a sample. It was 0.99 in all the 12 samples, indicating the obtained sequences represented the majority of bacteria sequences in all the 12 samples.

Table 2 Sampling depth and diversity indices for rectum, intestine, gill and water bacteria from three sites

	Rectum			Intestine			Gill			Water		
	RE-A	**RE-B**	**RE-C**	**IN-A**	**IN-B**	**IN-C**	**GI-A**	**GI-B**	**GI-C**	**W-A**	**W-B**	**W-C**
Sample depth												
No. of sequences	30,482	40,648	37,461	40,814	35,101	39,903	44,315	32,564	33,113	412,48	35,483	36,100
OTUs (97%)	422	389	383	298	288	225	125	140	88	438	434	461
Phylum	23	20	24	20	17	18	11	12	12	23	23	23
Class	45	39	44	37	32	31	22	23	18	46	47	45
Family	134	127	129	124	114	101	63	70	49	151	149	157
Genus	200	192	191	170	160	146	93	102	67	230	221	234
Diversity indices												
Chao 1	462.23	432.83	416.73	365.68	318.84	286.29	168.16	224	128.62	500.63	509.62	529.65
Shannon	3.63	2.85	2.89	2.13	2.84	2.24	0.74	1.12	0.46	3.91	4.05	4.21
Good's Coverage	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99

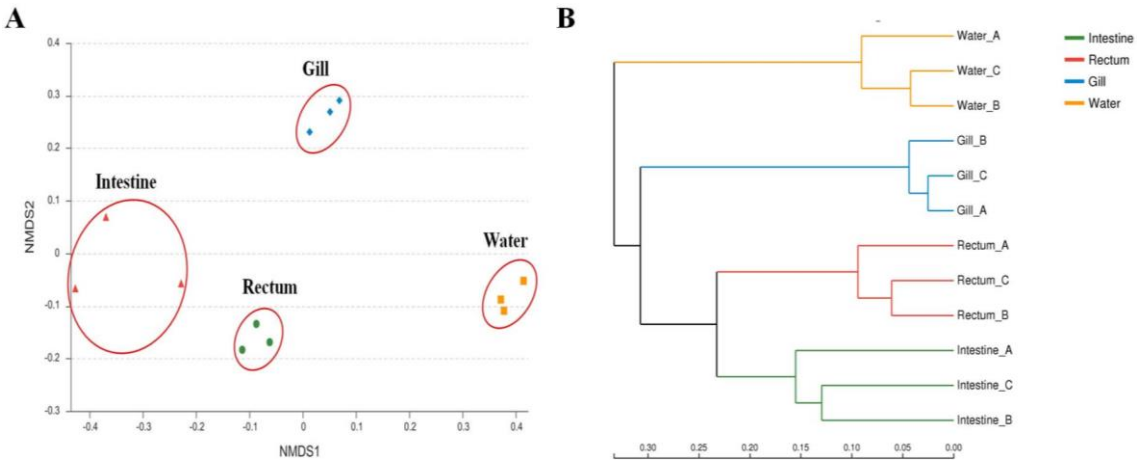

Fig. 1 Bacterial community dissimilarity among intestines, rectum, gills of Yesso scallop and its habitat waters. (A) Non-metric multidimensional scaling (NMDS) showing bacterial community difference in samples. The distances were determined using the weighted UniFrac method with relative abundance of OTUs. Circles indicate samples from intestines, triangles samples from rectum, diamonds samples from gills and squares samples from water. (B) Hierarchical clustering tree based on weighted UniFrac distances for sequences derived from Yesso scallop for each of the replicate intestines, rectum, gills and waters.

The bacterial diversity and richness were estimated by the Shannon index and Chao 1. The bacterial diversity in water group with Shannon index of 4.21 was higher than that of intestine, rectum and gill group (Shannon index = 2.84, 3.63 and 1.12, respectively). Chao 1 values were 462.23, 365.68, 168.16 and 529.7 in rectum, intestine, gill and water group, respectively (Table 2). It indicated that the bacterial richness was highest in the scallop inhabit waters. Alpha-diversity analysis showed that the bacterial diversity and richness in water sample were higher than that of intestines, rectum and gills, indicating the water harbored a remarkable diversity of bacterial communities.

The similarities and dissimilarities of bacterial community composition among all samples were explored by a NMDS (Fig. 1A) and hierarchical cluster analysis (Fig. 1B). The bacterial communities in intestine, rectum, gill and water replicates were clustered separately in different group on both axes in the NMDS analysis. The gill and water bacteria were separated on NMDS axis 2, but the intestine and rectum bacteria were clustered together. The clustering pattern in the samples did not change significantly among the sampling sites (Fig. 1A). In the hierarchical clustering tree based on bacterial community profiles at OTUs level, the bacterial communities from intestine, rectum, gill and water replicates formed four distinct clades. Intestine group was firstly clustered with rectum group and formed a sister group to the gill groups. The water group was separated from the scallop organ group and shared a closer relationship with gill groups (Fig. 1B). Venn diagram was constructed to evaluate the distribution of OTUs among different samples (Fig. 2). 108 OTUs were shared by all the samples. The number of specific OTUs in intestine, rectum, gill and water groups was 12, 32, 7 and 166, respectively.

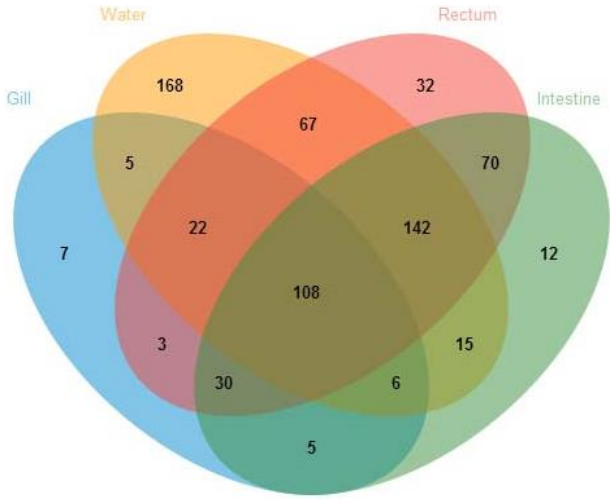

Fig. 2 Venn diagram analysis of the different samples. Venn diagram showing the shared and unique OTUs among intestines, rectum, gills of Yesso scallop and its habitat waters.

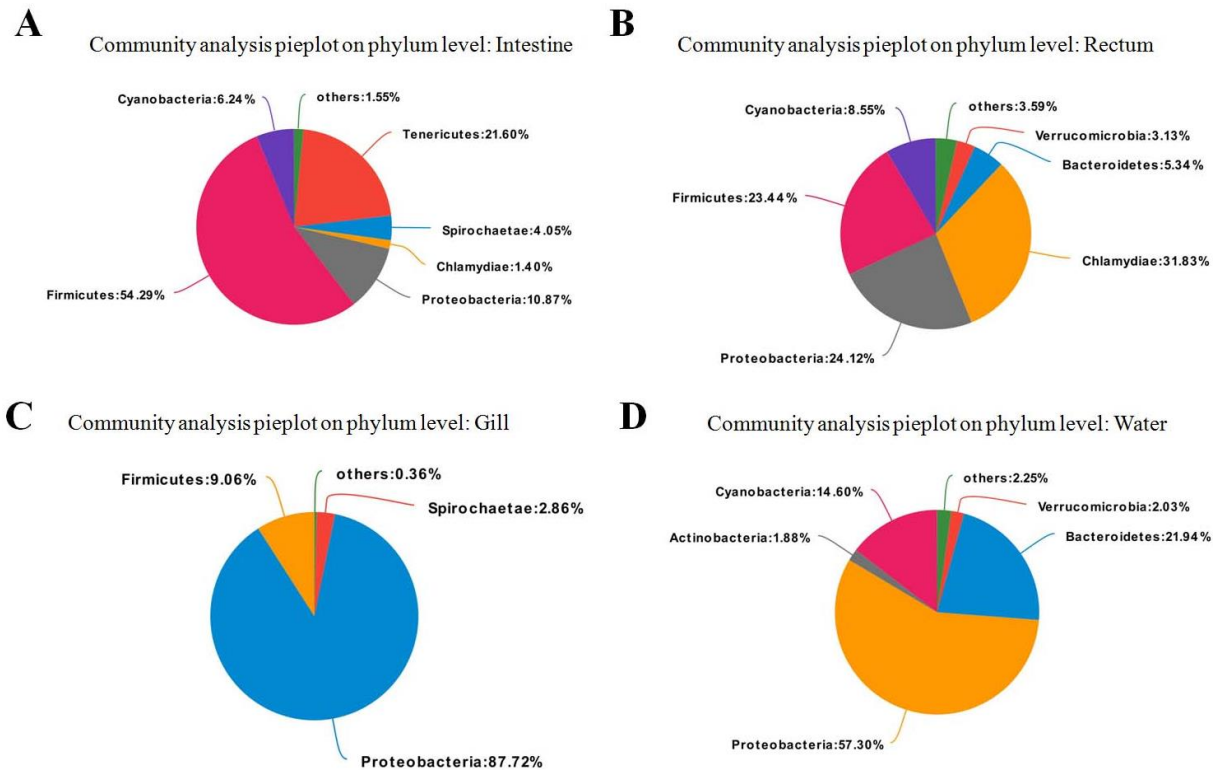

Fig. 3 Bacterial community compositions of intestines, rectum, gills of Yesso scallop and its habitat waters. Relative abundance of bacterial composition on phyla level. 'Other' represented a combination of species whose abundance was below 1 %.

Bacterial community compositions

The composition and abundance of bacterial communities in different samples was shown in Figure 3. At phylum level, the dominant bacteria in intestine were Firmicutes (54.28 %), Tenericutes (21.60 %) and Proteobacteria (10.87 %), which accounted for 86.75 % of total abundance (Fig. 3A). In rectum, three phyla of bacteria including Chlamydiae (31.83 %), Proteobacteria (24.12 %) and Firmicutes (23.44 %) were dominant, which accounted for 79.39 % of total abundance (Fig. 3B). Proteobacteria (87.73 %) and Firmicutes (9.05 %) were the dominant phyla in gill-associated bacteria, which accounted for 96.78 % of total abundance (Fig. 3C). Proteobacteria (57.47 %), Bacteroidetes (21.77 %) and Cyanobacteria (14.59 %) were dominant phyla in water accounting for 93.83 % of total abundance (Fig. 3D).

Further classification at family level indicated that bacterial communities varied considerably among different samples (Fig. 4). For instance, the intestine bacteria were dominated by Bacillaceae and Mycoplasmataceae belonging to the phyla Firmicutes and Tenericutes, respectively. Bacillaceae and Chlamydiales in the phyla Firmicutes and Chlamydiae were dominated in rectum. Hahellaceas and Bacillaceae were detected in all samples, but Hahellaceas showed more abundant in gill than that in the other groups. The proportions of Rhodobacteraceae, Cyanobacteria and Flavobacteriaceae, belonging to the phyla Proteobacteria, Cyanobacteria and Bacteroidetes, varied in waters, and Rhodobacteraceae was only detected in waters.

The abundance of the total bacteria and B. fragilis

The abundance of the total bacterial and *B. fragilis* of samples was examined by real-time quantitative PCR. It showed a high linear relationship between the C_t (threshold cycles) values and LogCN (denary logarithm of the copy numbers) in the standard curves of total bacteria load and *B. fragilis* (Fig. S2). The copy numbers of total bacterial load in waters ranged from 1.42×10^7 to 1.99×10^7 copies per nanogram of DNA, which was significantly higher than that in other groups. The relatively lower copy numbers of total bacterial load were observed in rectum, which was about 22.28 % of total bacterial load in waters (Fig. 5A). Whereas the copy numbers of the *B. fragilis* in rectum ranged from 5.67×10^3 to 6.43×10^3 copies per nanogram of DNA, which was significantly higher than eleven times and two times that in intestine and gill, respectively (Fig. 5B).

Discussion

The Yesso scallop is a filter-feeding marine bivalve, which filter and accumulate large numbers of microbe from the harvesting water (Antunes *et al.*,

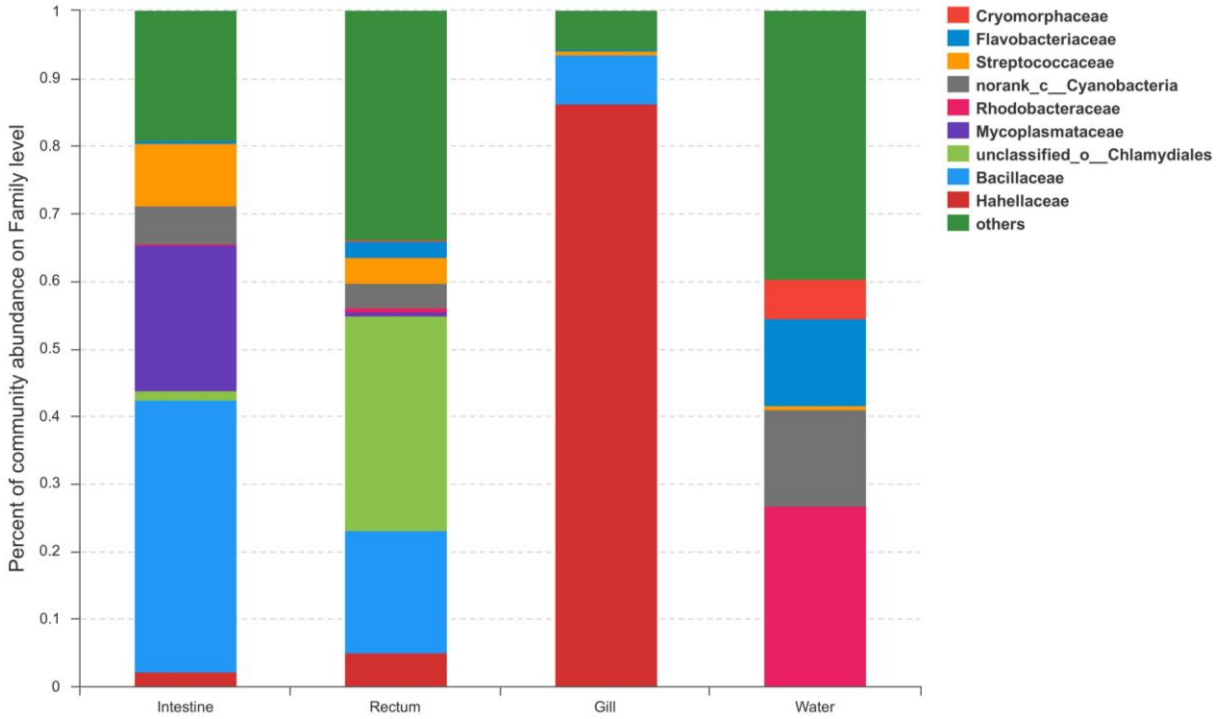

Fig. 4 Bacterial community compositions at family level in intestines, rectum, gills of Yesso scallop and its habitat waters. 'Other' represented a combination of species whose abundance was below 5 %.

2010). Bacteria can exert positive or negative influences on the health status of aquatic animals, either by the construction of symbiotic relationships or by causing diseases, respectively (Antunes *et al*., 2010). The symbiotic microbiota has been investigated in many marine invertebrates, such as Easter oyster, scallop, Black Tiger Shrimp and sea cucumber (King *et al*., 2012; Coton *et al*., 2013; Rungrassamee *et al*., 2013; Gao *et al*., 2014). However, the information about the symbiotic microbiota in *P. yessoensis* and the microbiota present in its habitat waters is still very limited. In the present study, bacterial composition inside and outside the body of Yesso scallop was analyzed. The objectives of this work were to identify microbiota diversity in different functional organs (intestine, rectum and gill) of *P. yessoensis* and to assess the relationship between the environmental microbiota and symbiotic microbiota, which could contribute to better understanding about the microbiota structure inside and outside the body of Yesso scallop also, and also provided critical information to develop strategies of disease prevention and probiotic application in scallop aquaculture.

In aquatic ecosystems, aquatic animals are in direct contact with the environment water. Therefore, they are in continual contact with a complex and dynamic microbiota, and some of them are host symbiotic microbiota (Gatesoupe, 1999). The temperature of the environmental waters was a key driver of bacterial community, the higher temperatures harbored remarkably higher diversity and richness in bacterial composition when compared to the lower temperatures (Tang *et al*., 2014). Marine microbial have seasonal dynamic changes in the south sea of Korea (Suh *et al*., 2015). During the sampling period, temperature recorded values close to 12 °C in the surface layers, and the lower water temperature may be affected the bacterial community inside and outside the body of Yesso scallop. Although intestinal bacteria of aquatic animals may mainly originate from the culture waters (Han *et al*., 2010; Wu *et al*., 2012), the symbiotic microbiota of some aquatic animals showed significant difference from that in the surrounding environment (Suh *et al*., 2015; Zhang *et al*.,2016). For example, clear differences were seen between the bacterial community associated with great scallop (*Pecten maximus*) and in the water from the different aquaculture systems (Sandaa *et al*., 2003). In the present study, Proteobacteria was found to be the dominant phyla in the organs of Yesso scallop and its habitat waters, which was consistent with previous reports (Wu *et al*., 2012; Zhang *et al*., 2016). But the bacterial community structures in waters and organs of Yesso scallop were not exactly the same. The bacteria belonging to Rhodobacteraceae family was dominant in water but almost absent in intestine, rectum and gill samples, suggesting that there were specific microbiota for organs and habitat waters of aquatic animal. It was suggested that bivalve could select bacteria through filtration by gills and remove bacteria through their immune system (Antunes *et al*., 2010; Meisterhans *et al*., 2016). For example, the wood-eating marine bivalve shipworms can select

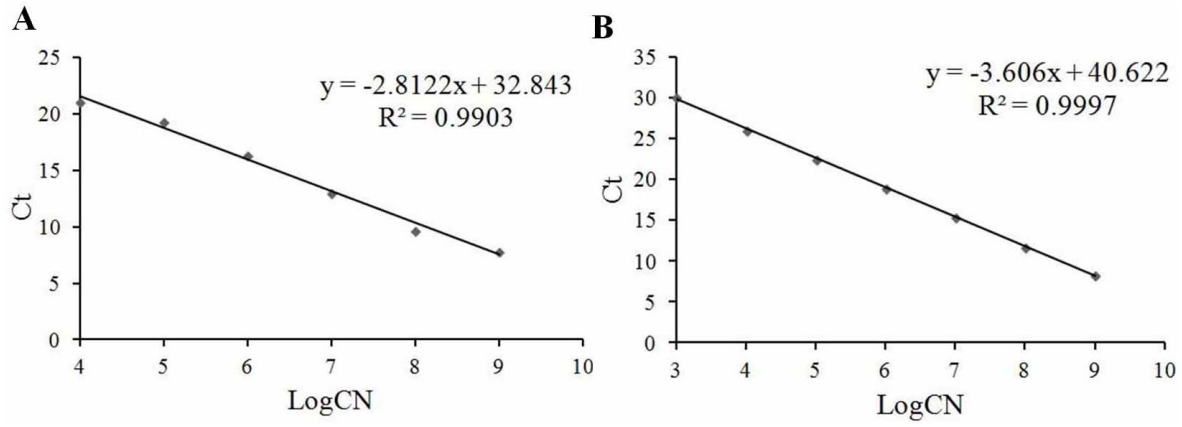

Fig. S2 Standard curve of the 16S rDNA gene copy number of the total bacteria (A) and *B. fragilis* load (B), showing the linear relationship between the threshold cycles (C_t) and copy number.

endosymbiotic bacteria by gills to produce wood degrading enzymes (O'Connor *et al*., 2014). *Anodonta cygnea* has the ability to filter and eliminate *Escherichia coli* from the surrounding environment, and establish a commensal relationship with *Vibrio metschnikovii* and *Aeromonas sobria* through phagocytic process conducted by heamolymph circulating cells without causing disease and mortality (Antunes *et al*., 2010). The persistence of some bacteria in the bivalve tissue depends in part on their resistance to the bactericidal activity of the hemolymph (Canesi *et al*., 2001; Zampini *et al*., 2003). In addition, the bacterial diversity and abundance of the inhabit waters of Yesso scallop were significantly higher than that of its intestines, rectum and gills, which was similar to the previous reports in scallop, fish and sea cucumber (Sandaa *et al*., 2003; Gao *et al*., 2014; Miyake *et al*., 2015), suggesting that most of the bacterial species in aquatic animal intestines might live as specialized members of symbiotic microbiota rather than free-living environmental bacteria. Bivalves depend on their immune system to eliminate the invading bacteria and decrease the microbial load inside their bodies (Antunes *et al*., 2010). The symbiotic microbiota of aquatic animalsis derived from the habitat waters. The difference between the symbiotic microbiota and environmental bacteria suggests a mechanism of selection, adaptation and regulation between the host and the microbe.

The microbiota structure differed among organs indicated that host have body site specific microbiota (Zhang *et al*., 2016). The different organs in Yesso scallop (intestine, rectum and gill) represent bacterial niches with unique feature. Recently, the microbiota associating with Great scallop (*Pecten maximus*) gonads (Lasa *et al*., 2014) and the bacterial diversity in the mantle of healthy and incised symptoms of Yesso scallop (*P. yessoensis*) (Ding *et al*., 2014) have also been studied. In Manila clam, the bacterial communities among organs were different, which was associated with the respective physiological functions of organs (Meisterhans *et al*., 2016). The differences in intestine regional structures of adult mussel (*Mytilus edulis*), such as intestinal epithelium and mucous cells, contribute to the functional differences (Fritsch *et al*., 1976). In Norway lobster (*Nephrops norvegicus*), the digestive system consists of the foregut, midgut and hindgut. The midgut is the main absorption organ, and no absorption occurs in the short hindgut (Yonge, 1924; Miyake *et al*., 2015). Previous studies revealed the functional differences between midgut and hindgut of the *Eriocheir sinensis*, which affacted the diversity and abundance of bacteria in different intestinal regions (Chen *et al*., 2015). In the present study, significant differences were observed in the rectum and intestines associated bacteria, which was similar to the autochthonous microbiota in different parts of adult yellow grouper digestive tract (Zhou *et al*., 2009). These results suggested that the bacterial niche with unique features in different intestinal structures might affect physiological functions, and the microbiota in rectum and intestines should experience district selective processes for their microbial community. The variations of intestine microbiota can indicate the alternations of food supply for marine invertebrates (Meziti *et al*., 2010; Miyake *et al*., 2015). Gills can perform physical and physiological functions to selectively exclude microbe, therefore, the microbiota communities in gills and intestines are always different (Wildish, 1998). It has been reported that the microbe associated with bivalve organs might be partially selected through phagocytosis mechanisms (Pruzzo *et al*., 2005). In the present study, the different microbiota patterns among organs also indicated a selection of scallop microbiota at organ scale.

B. fragilis is a gram-negative anaerobic bacterium found in the intestinal tract of most animals and human (Betteken *et al*., 2015). It is the major component of the endogenous human bowel flora, commonly related to a variety of human infections, such as bacteremia and wound infection (Cheng *et al*., 2009; Martin *et al*., 2009), and contributes significantly to the morbidity and even mortality (Hadano *et al*., 2013). In mub crabs, *B. fragilis*

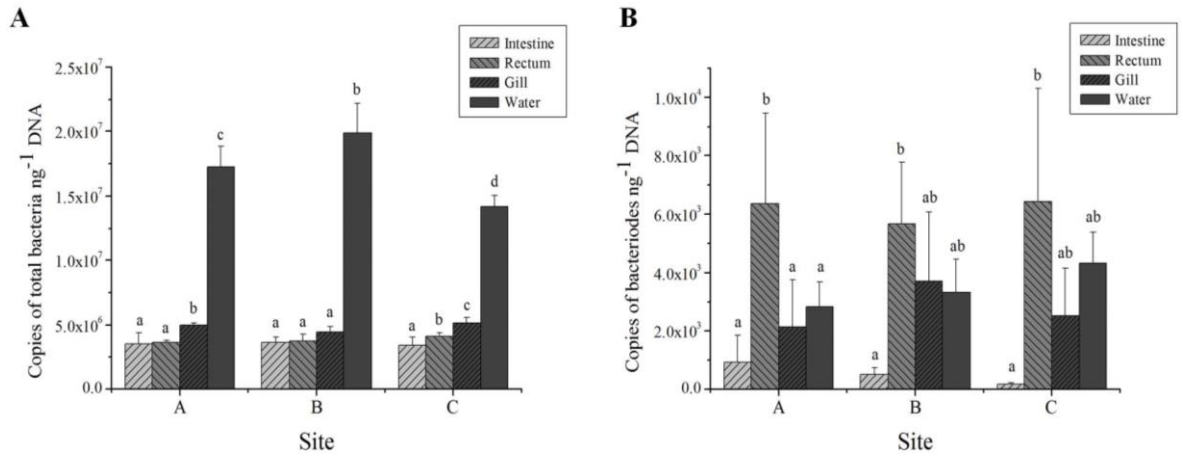

Fig. 5 The 16S rDNA gene copy number of the total bacteria (A) and *B. fragilis* load (B) by real-time quantitative PCR inintestines, rectum and gills of Yesso scallop and its habitat waters with three parallel sites.

was present in the intestine of both healthy and diseased individuals (Li*e et al*., 2012). Bacteroidetes was also found to be common in wild and pond-raised crab intestines, but the abundance of total bacterial and *B. fragilis* load in the pond-raised crabs were about four-to-ten times higher than that in wild crabs (Li *et al*., 2007a). Bacteroidetes were dominant bacteria in the adductor muscle of sick Yesso scallop (*P. yessoensis*), but barely in the adductor muscle of healthy scallop, indicating it might be related to the disease of scallops (Dou *et al*., 2016). *B. fragilis* has been reported to activate the TLR pathway in T lymphocytes to establish host microbial symbiosis (Round *et al*., 2011). In the present study, the abundance of Bacteroidetes and *B. fragilis* in rectum was much higher than that in gills and intestines, indicating the abundance of *B. fragilis* in rectum might attribute to the scallop intestinal disease. It is suggested that a small amount of *B. fragilis* cannot cause scallop rectum disease, and there should be a threshold of intestinal microbiota imbalance for the outbreak of intestinal disease. In addition, the proportion of Bacteroides decreased significantly from spring to summer, and increased during autumn and highest in winter in the south sea of Korea (Suh *et al*., 2015). The bacterial community composition in the digestive diverticula of scallops (*Chlamys farreri*) was significantly seasonal variation (Yang *et al*., 2012). The bacterial community changed significantly at different development stages of Yesso Scallop larvae (*P. yessoensis)* (Sun *et al*., 2016). To a certain extent, the high abundance of *B. fragilis* in the rectum may be affected by the water microbiota, seasons, scallop development stages.

In conclusion, intestine, rectum and gill of Yesso scallop and its habitat waters have distinct microbiota. The bacterial diversity and richness in waters were higher than that in intestines, rectum and gills, suggesting that the bacterial community in the body of scallop was more specialized than that in its habitat waters. The different microbiota patterns among scallop organs indicated that there was a selection of microbiota at organ scale. The results provided useful information for the disease prevention and probiotic application in scallop aquaculture.

Acknowledgement

This study was supported by earmarked fund (CARS-48) from Modern Agro-industry Technology Research System, a grant from National Science Foundation of China (No. 31530069), Dalian high level talent innovation support program (2015R020), and the Research Foundation for Talented Scholars in Dalian Ocean University (to L S).

References

Aide L, Alex M, Camelo-Castillo A, Belda-Ferre P, Romalde JL. Analysis of the scallop microbiota by means of 16S rRNA gene pyrosequencing. Front. Mar. Sci., 2014.

Antunes F, Hinzmann M, Lopes-Lima M, Machado J, Costa PMD. Association between environmental microbiota and indigenous bacteria found in hemolymph, extrapallial fluid and mucus of *Anodonta cygnea* (Linnaeus, 1758). Microb. Ecol. 60: 304-309, 2010.

Betteken MI, Rocha ER, Smith CJ. Dps and DpsL mediate survival *in vitro* and *in vivo* during the prolonged oxidative stress response in *bacteroides fragilis*. J. Bacteriol. 197: 3329-38, 2015.

Canesi L, Pruzzo C, Tarsi R, Gallo G. Surface interactions between *Escherichia coli* and hemocytes of the Mediterranean mussel *Mytilus galloprovincialis* Lam. leading to efficient bacterial clearance. Appl. Environ. Microbiol. 67: 464-468, 2001.

Cardenas CA, Bell JJ, Davy SK, Hoggard M, Taylor MW. Influence of environmental variation on symbiotic bacterial communities of two temperate sponges. FEMS Microbiol. Ecol. 88: 516, 2014.

Chao A, Lee S. Estimating the number of classes via sample coverage. J. Amer. Statistical Assoc. 87: 210-217, 1992.

Chen X, Di P, Wang H, Li B, Pan Y, Yan S, *et al*.

Bacterial community associated with the intestinal tract of Chinese mitten crab (*Eriocheir sinensis*) farmed in Lake Tai, China. PLOS ONE 10: e0123990, 2015.

Cheng CW, Lin HS, Ye JJ, Yang CC, Chiang PC, Wu TS, *et al.* Clinical significance of and outcomes for *Bacteroides fragilis* bacteremia. J. Microbiol. Immunol. Infect. 42: 243-250, 2009.

Coton M, Joffraud JJ, Mekhtiche L, Leroi F, Coton E. Biodiversity and dynamics of the bacterial community of packaged king scallop (*Pecten maximus*) meat during cold storage. Food Microbiol. 35: 99-107, 2013.

Ding J, Dou Y, Xu GR, Wang YN, Chang YQ. Bacterial diversity in the mantle of *Patinopecten yessoensis* revealed by 454 pyrosequencing. Chinese J. Appl. Ecol. 25: 3344-3348, 2014.

Dou Y, Zhao XW, Ding J, Liu SC, Zhang T. Application of high-throughput sequencing for analyzing bacterial diversity in the adductor muscle of sick and healthy *Patinopecten yessoensis*. Chinese J. Ecol. 2016.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 27: 2194-2200, 2011.

Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods. 10: 996, 2013.

Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. USA 103: 626, 2006.

Fritsch HA, Van NS, Pearse AG. Cytochemical and immunofluorescence investigations of insulin-like producing cells in the intestine of *Mytilus edulis* L. (Bivalvia). Cell Tissue Res. 165: 365-369, 1976.

Gao F, Tan J, Sun H, Yan J. Bacterial diversity of gut content in sea cucumber (*Apostichopus japonicus*) and Its Habitat Surface Sediment. J. Ocean Univ. China 13: 303-310, 2014.

Gatesoupe F. The use of probiotics in aquaculture. Aquaculture. 180: 147-165, 1999.

Goffredi SK, Gregory A, Jones WJ, Morella NM, Sakamoto RI. Ontogenetic variation in epibiont community structure in the deep-sea yeti crab, Kiwa puravida: convergence among crustaceans. Mol. Ecol. 23: 1457-1472, 2014.

Goodfellow M, Stackebrandt E. Nucleic acid techniques in bacterial systematics. J. Wiley,1991.

Hadano Y, Iwata H. Case report: Septic thrombophlebitis of the superior mesenteric vein with bacteraemia caused by *Bacteroides fragilis* and *Streptococcus intermedius* as a complication of diverculitis. Bmj. Case. Rep. 2013.

Han S, Liu Y, Zhou Z, He S, Cao Y, Shi P, *et al*. Analysis of bacterial diversity in the intestine of grass carp (*Ctenopharyngodon idellus*) based on 16S rDNA gene sequences. Aquacult. Res. 42: 47-56, 2010.

Hou R, Bao Z, Wang S, Su H, Li Y, Du H, *et al*. Transcriptome sequencing and de novo analysis for Yesso scallop (*Patinopecten yessoensis*) using 454 GS FLX. PLOS ONE 6: e21560, 2011.

King GM, Judd C, Kuske CR, Smith C. Analysis of stomach and gut microbiomes of the eastern oyster (*Crassostrea virginica*) from coastal Louisiana, USA. PLOS ONE 7: e51475, 2012.

Klaus JS, Friaslopez J, Bonheyo GT, Heikoop JM, Fouke BW. Bacterial communities inhabiting the healthy tissues of two Caribbean reef corals: interspecific and spatial variation. Coral Reefs 24: 129-137, 2005.

Lafferty KD, Porter JW, Ford SE. Are diseases increasing in the ocean? Annu. Rev. Ecol. Evol. S. 35: 31-54, 2004.

Lai Y, Di NA, Nakatsuji T, Leichtle A, Yang Y, Cogen AL, *et al*. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. Nat. Med. 15: 1377-1382, 2009.

Larsen AM, Mohammed HH, Arias CR. Characterization of the gut microbiota of three commercially valuable warmwater fish species. J. Appl. Microbiol. 116: 1396-1404, 2014.

Li K, Guan W, Wei G, Liu B, Xu J, Zhao L, *et al*. Phylogenetic analysis of intestinal bacteria in the Chinese mitten crab (*Eriocheir sinensis*). J. Appl. Microbiol. 103: 675-682, 2007a.

Li K, Zheng TL, Tian Y, Yuan JJ. Bacterial community structure in intestine of the white shrimp, *Litopenaeus vannamei*. Acta Microbiol. Sin. 47: 649-653, 2007b.

Li S, Sun L, Wu H, Hu Z, Liu W, Li Y, *et al*. The intestinal microbial diversity in mud crab (*Scylla paramamosain*) as determined by PCR-DGGE and clone library analysis. J. Appl. Microbiol. 113: 1341-1351, 2012.

Li W, Xue Z. Healthy sustainable proliferation and cultivation of scallop *Patinopecten yessoensis*. Fish. Sci. 9: 018, 2005.

Liu C, Song Y, McTeague M, Vu AW, Wexler H, Finegold SM. Rapid identification of the species of the*Bacteroides fragilis*group by multiplex PCR assays using group- and species-specific primers. FEMS Microbiol. Lett. 222: 9-16, 2003.

Liu H, Wang L, Liu M, Wang B, Jiang K, Ma S, *et al*. The intestinal microbial diversity in Chinese shrimp (*Fenneropenaeus chinensis*) as determined by PCR-DGGE and clone library analyses. Aquaculture 317: 32-36, 2011.

Liu R, Qiu L, Zhao X, Zhang H, Wang L, Hou Z, *et al*. Variation analysis of pathogenic *Vibrio spp.* and *Pseudomonas spp*. in Changhai mollusc farming waters using real-time PCR assay during 2011-2014. Mar. Biol. Res. 12: 146-157, 2016.

Lu F, Liu M, Simister R, Webster NS, Thomas T. Marine microbial symbiosis heats up: the phylogenetic and functional response of a sponge holobiont to thermal stress. Isme J. 7: 991-1002, 2013.

Luo P, Hu C, Xie ZY, Zhang LP, Ren CH, Xu YH. PCR-DGGE analysis of bacterial community composition in brackish water *Litopenaeus vannamei* culture system. J. Trop. Oceanogr. 25: 49-53, 2006.

Luo P, Hu C, Zhang LP, Ren CH. PCR-DGGE analysis of bacterial communities in marine *Litopenaeus vannamei* culture system. J. Fish Sci. China, 2009.

Martin T, Aziz H. *Bacteroides fragilis*: a case study of bacteremia and septic arthritis. Clin. Lab. Sci.

22:131-135, 2009.
Meisterhans G, Raymond N, Girault E, Lambert C, Bourrasseau L, Montaudouin XD, *et al*. Structure of manila clam (*Ruditapes philippinarum*)microbiota at the organ scale in contrasting sets of individuals. Microb. Ecol. 71: 194, 2016.
Meziti A, Ramette A, Mente E, Kormas KA. Temporal shifts of the Norway lobster (*Nephrops norvegicus*) gut bacterial communities. FEMS Microbiol. Ecol. 74: 472-484, 2010.
Miyake S, Ngugi DK, Stingl U. Diet strongly influences the gut microbiota of surgeonfishes. Mol. Ecol. 24: 656, 2015.
Ngangbam AK, Baten A, Waters DL, Whalan S, Benkendorff K. Characterization of bacterial communities associated with the Tyrian purple producing gland in a marine gastropod. PLOS ONE 10: e0140725, 2015.
Nielsen SJ, Harder T, Steinberg PD, Sea urchin larvae decipher the epiphytic bacterial community composition when selecting sites for attachment and metamorphosis, FEMS Microbiol. Ecol. 91: 1-9, 2015.
O'Connor RM, Fung JM, Sharp KH, Benner JS, McClung C, Cushing S, *et al.* Gill bacteria enable a novel digestive strategy in a wood-feeding mollusk. Proc. Natl. Acad. Sci. USA 111: E5096-104, 2014.
Plowright RK, Sokolow SH, Gorman ME, Daszak P, Foley JE. Causal inference in disease ecology: investigating ecological drivers of disease emergence. Front. Ecol. Environ. 6: 420-429, 2008.
Prosser CL, Nystrom RA, Nagai T. Electrical and mechanical activity in intestinal muscles of several invertebrate animals. Comp. Biochem. Physiol. 14: 53-70，1965.
Pruzzo C, Gallo G, Canesi L. Persistence of vibrios in marine bivalves: the role of interactions with haemolymph components. Environ. Microbiol. 7: 761-772, 2005.
Rawls JF, Samuel BS, Gordon JI. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. Proc. Natl. Acad. Sci. USA 101: 4596, 2004.
Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, *et al*. The Toll-like receptor pathway establishes commensal gut colonization. Science 332: 974-977, 2011.
Ruby E, Henderson B, Mcfall-Ngai M. Microbiology. We get by with a little help from our (little) friends. Science 303: 1305-1307, 2004.
Rungrassamee W, Klanchui A, Chaiyapechara S, Maibunkaew S, Tangphatsornruang S, Jiravanichpaisal P, *et al*. Bacterial population in intestines of the black tiger shrimp (*Penaeus monodon*) under different growth stages. PLOS ONE 8: e60802, 2013.
Sandaa RA, Magnesen T, Torkildsen L, Bergh O. Characterisation of the bacterial community associated with early stages of Great Scallop (*Pecten maximus*), using denaturing gradient gel electrophoresis (DGGE). Syst. Appl. Microbiol. 26:302-311, 2003.
Shannon CE. A mathematical theory of communication. Bcl Syst. Tech. J. 1948.
Suh SS, Park M, Hwang J, Kil EJ, Jung SW, Lee S,*et al*. Seasonal dynamics of marine microbial community in the south sea of korea. PLOS ONE 10: e0131633, 2015.
Sun X, Liu JC, Li M, Zhao XW, Liang J, Sun PH, *et al*. Characterization of bacterial communities associating with larval development of Yesso Scallop (*Patinopecten yessoensisis* Jay, 1857) by high-throughput sequencing. J. Ocean Univ. China 15: 1067-1072, 2016.
Sweet MJ, Croquer A, Bythell JC. Bacterial assemblages differ between compartments within the coral holobiont. Coral Reefs 30: 39-52, 2011.
Tang Y, Tao P, Tan J, Mu HZ, Peng L, Yang DD, *et al*. Identification of bacterial community composition in freshwater aquaculture system farming of *Litopenaeus vannamei* reveals distinct temperature-driven patterns. Int. J. Mol. Sci. 15:13663, 2014.
Wang M, Ahrné S, Jeppsson B, Molin G. Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. FEMS microbiol. Ecol. 54: 219-231, 2005.
Wang Q. Introduction of Japanese scallop and prospect of culture it in northern China. Fish. Sci. 3: 24-27, 1984.
Wildish DJ. Ecology of marine bivalves: an ecosystem approach, Limnology Oceanography, 43, 1998.
Wu S, Wang G, Angert ER, Wang W, Li W, Hong Z, *et al*. Composition, diversity, and origin of the bacterial community in grass carp intestine. PLOS ONE 7: e30440, 2012.
Xiao J, Ford SE, Yang H, Zhang G, Zhang F, Guo X. Studies on mass summer mortality of cultured zhikong scallops (*Chlamys farreri* Jones et Preston) in China. Aquaculture 250: 602-615, 2005.
Xu J, Gordon JI. Honor thy symbionts. Proc. Natl. Acad. Sci. USA 100: 10452-10459, 2003.
Yan Q, Cj VDG, Yu Y. Bacterial community assembly and turnover within the intestines of developing zebrafish. PLOS ONE 7: e30603, 2012.
Yang CX, Li Y, Zhang JY, Wang CM. Seasonal changes of bacterial community composition in digestive diverticula of scallops *Chlamys farreri*. J. Fisheries China 36: 1579-1584, 2012.
Ye L, Amberg J, Chapman D, Gaikowski M, Liu WT. Fish gut microbiota analysis differentiates physiology and behavior of invasive Asian carp and indigenous American fish. Isme J. 8: 541, 2013.
Yonge C. Studies on the comparative physiology of digestion. J. Exp. Biol. 1: 343-389, 1924.
Yuan X, Zhang M, Liang Y, Liu D, Guan D. Self-pollutant loading from a suspension aquaculture system of Japanese scallop (*Patinopecten yessoensis*) in the Changhai sea area, Northern Yellow Sea of China. Aquaculture 304: 79-87，2010.
Zampini M, Canesi L, Betti M, Ciacci C, Tarsi R, Gallo G, *et al*. Role for mannose-sensitive hemagglutinin in promoting interactions between Vibrio cholerae El Tor and mussel

hemolymph. Appl. Environ. Microbiol. 69: 5711-5715,2003.

Zhang M, Sun Y, Chen L, Cai C, Qiao F, Du Z, *et al*. Symbiotic bacteria in gills and guts of chinese mitten crab (*Eriocheir sinensis*) differ from the free-living bacteria in water. PLOS ONE 11: e0148135, 2016.

Zhou Z, Liu Y, Shi P, He S, Yao B, Ringø E. Molecular characterization of the autochthonous microbiota in the gastrointestinal tract of adult yellow grouper (*Epinephelus awoara*) cultured in cages. Aquaculture 286: 184-189, 2009.