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RESEARCH REPORT

The temporal dynamics of bacteria in the coelomic fluid of sea cucumber *Apostichopus japonicus* after evisceration

C Zhang[1], Z Yu[2,3,4,5], Z Xue[2,3,4,5], H Li[3,4,5], J Zhu[1], L Wang[2,3,4,5], L Song[2,3,4,5]*

[1]School of Marine Sciences, Ningbo University, Ningbo 315211, China
[2]Laboratory of Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266235, China
[3]Liaoning Key Laboratory of Marine Animal Immunology, Dalian Ocean University, Dalian 116023, China
[4]Liaoning Key Laboratory of Marine Animal Immunology & Disease Control, Dalian Ocean University, Dalian 116023, China
[5]Dalian Key Laboratory of Aquatic Animal Disease Prevention and Control, Dalian Ocean University, Dalian 116023, China

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Abstract

Sea cucumbers have been emerged as important models to study organ regeneration and development owing to the capacity to regenerate its organs quickly after evisceration. Evisceration is a special defense mechanism for sea cucumber to eject all of internal organs when they encounter predators or adverse environmental conditions. However, little was known about the dynamics of bacterial community in coelomic fluid after evisceration. In the present study, evisceration was induced by intracelomic injection of 0.35 M KCl, and the significantly alternation of bacterial community in coelomic fluid of sea cucumber *Apostichopus japonicus* was observed with lower diversity and total bacterial load at 7 dpe (days post evisceration) and 14 dpe. The bacterial community was tended to restore at 28 dpe. In particular, relative abundances of Bacteroidetes and Rubritaleaceae, which involved in degradation of polysaccharides and lipid, increased significantly at 7 dpe ($p < 0.05$), and returned to the original level at 28 dpe. In addition, the predicted functions of bacterial community indicated that the bacteria associated with metabolism pathways of amino acid, lipid and carbohydrate also increased significantly at 7 dpe. These results suggested that the bacterial community in coelomic fluid of *A. japonicus* was highly dynamic and could rebuild a stable community structure after evisceration. It was suggested that the enriched metabolic related beneficial bacteria at early stage played a role after evisceration in terms of decomposing polysaccharides and lipid to provide energy.

Key Words: sea cucumber; evisceration; coelomic fluid; bacterial community

Introduction

Evisceration is a special physiological phenomenon in sea cucumbers that all the internal organs, including coelomic fluid, coelomocytes, intestinal tract and respiratory trees can be ejected when they encounter adverse environmental conditions or pathogenic infection (Li *et al*., 2018). The evisceration can be induced artificially and the sea cucumbers display a capacity to regrow body parts and internal organs after evisceration (Emson and Wilkie, 1980), which is much greater than that of sea stars and sea urchins, making them prime regeneration models (Carnevali, 2006). In the past decades, there has been increasing reports on the mechanisms of intestinal regenerative processes in sea cucumbers, including morphological features, cell division, dedifferentiation, cell proliferation and migration, nerve regrowth, and molecular regulation (García-Arrarás *et al*., 1998; Dolmatov and Ginanova, 2009; Sun *et al*., 2011; Sun *et al*., 2013; Li *et al*., 2017b; Zhang *et al*., 2017). The regeneration of internal organs in sea cucumber is always accompanied with the reconstruction of host's microbiota, and the quantity and structure of microbial community is suggested to be linked with the development of regenerating intestine (Zhang *et al*., 2020). Understanding the dynamics of microbial community after evisceration of sea cucumber is helpful to investigate the interaction

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

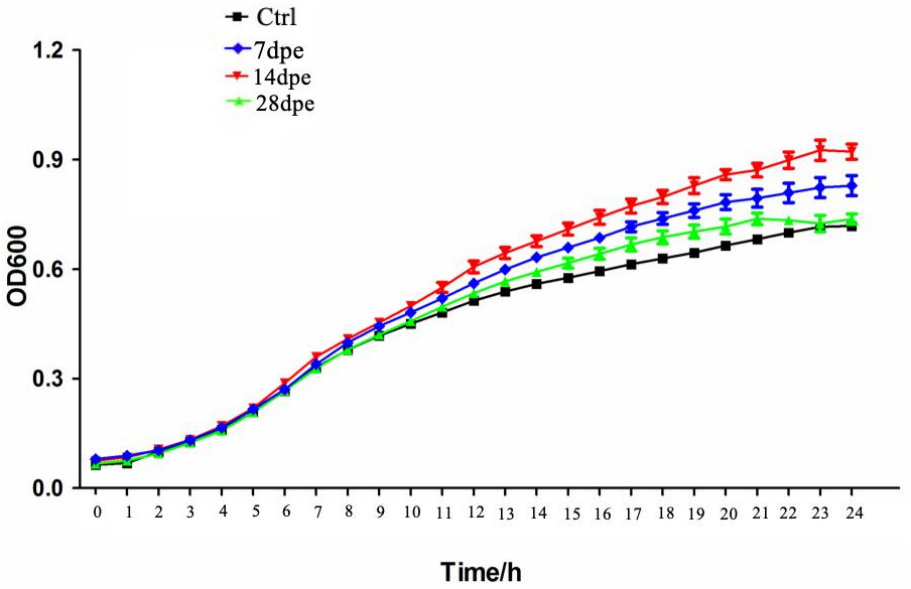

Fig. 1 The growth curves of *V. splendidus* after incubation with coelomic fluids of different post-evisceration groups

between microbiota and organ development in sea cucumber.

Accumulating evidence proves that microbiota play important roles in animal survival, homeostasis and development (McFall-Ngai *et al*., 2013). In mammals, the symbiotic microbiota have been considered to be a key element for intestinal regeneration and hematopoiesis. For example, the microbiota-intestinal stem cells dialog is a key element for intestinal regeneration in human (Stedman *et al*., 2016), and the hematopoiesis can be promoted by microorganisms in mice (Khosravi *et al*., 2014). The microbiota is also essential for organ regeneration in invertebrate. It has been proved that the dramatic change of microbiome in the coelomic of freshwater flatworm *Schmidtea mediterranea* can cause the loss of its regenerative capacity (Arnold *et al*., 2016), and the structure of microbial community was found to be linked with the development of regenerating intestine in *A. japonicus* (Wang *et al*., 2018; Zhang *et al*., 2019; Zhang *et al*., 2020). However, the information about the changes of bacterial community in coelomic fluid after evisceration is very limited, and the data of coelomic fluid microbiota in sea cucumber are mainly obtained by means of culture-dependent methods. With the rapid development of modern biotechnology, some molecular fingerprinting methods have been applied to provide realistic estimates of community diversity and composition. Next-generation sequencing (NGS) is commonly used for the detailed characterization of microbial community composition and dynamics, even for the rare phylotypes. The results of NGS can act as a seed bank to figure out the microbial community response to environmental change, and NGS method has been employed to characterize the bacterial communities in lower invertebrates, including sea cucumber gut microbiome (Zhang *et al*., 2018).

Previous studies have demonstrated that the host can develop tolerance to microbial community and maintain a mutually beneficial relationship with the microbiota (Knoop *et al*., 2017). Adult microbiota are thought to be relatively stable over time, and this stability imparts resilience to various perturbations, ensuring the host's continued function (Koenig *et al*., 2011). In the present study, high-throughput sequencing of 16S rRNA gene was employed to study the temporal dynamics of bacteria in coelomic fluid of *A. japonicus* after evisceration with the objectives to characterize the changes of bacterial community structure in coelomic fluid after evisceration, and predict the potential roles of coelomic fluid bacterial, which could help for the further evaluation on the roles of bacterial community after evisceration.

Materials and methods

Experimental design and sample collection

Healthy adult sea cucumbers about 100 ± 10 g in weight were obtained from a commercial farm in Dalian, Liaoning province, and cultured in natural seawater at 10 - 12 °C under natural illumination. Evisceration was induced by intracoelomic injection of 1.5 mL of 0.35 M KCl. Previous reports indicated that the lumen formation of the new intestine began at 7 dpe, and the intestine gradually developed to form a complete structure at 14-21 dpe (Sun *et al*., 2011; Sun *et al*., 2013), while the regeneration of coelomocytes was basically completed at 28 dpe (Li *et al*., 2018). Nine individuals of *A. japonicus* were sampled at four time points, including pre-evisceration (used as control group), 7 dpe (days post-evisceration), 14 dpe, and 28 dpe. For coelomic fluid collect, sea cucumber was allowed to stand on paper to desiccate the seawater accumulated in the body. The ventral surface of *A.*

japonicus was opened longitudinally and the coelomic fluid was collected in a petri dish. The coelomic fluid from three individuals was pooled together, and there were three replicates for each time point. The coelomic fluid was filtered through a 300-mesh bolting cloth under sterile conditions to remove turbid impurities, and then transferred to sterile microfuge tubes and stored at -80 °C before genomic DNA extraction.

Antibacterial assay of coelomic fluid at different time post-evisceration

The antibacterial activities of coelomic fluids against *Vibrio splendidus* at different time after evisceration were tested as previously reported (Jia *et al*., 2015). *V*. *splendidus* was cultured in 2216E liquid medium (5 g/L peptone and 1 g/L yeast extract in seawater). The culture in logarithmic phase was centrifuged, washed by TBS (20 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.5) for three times, and then resuspended in TBS to the final concentration of 10^4 CFU/mL. Fifty microliters of coelomic fluid were mixed with the same volume of *V*. *splendidus* resuspension and incubated at room temperature for 1 h. Twenty microliters of the mixtures were added into a 96-well microliter plate with 200 μL 2216E culture medium. The plate was placed in a microplate reader (Bioteka, USA) at 28 °C with a shake for every five seconds. Absorbance value at OD_{600} was measured every 30 minutes for 24 h to detect the growth curve. Each group was repeated for three times.

DNA extraction and amplicon sequencing

DNA was extracted from 12 whole coelomic fluid samples with E.Z.N.A Soil DNA kit (OMEGA, USA) according to the manufacturer's protocol. The concentration and purity of DNA samples were examined by 1 % agarose gel electrophoresis and a Nanodrop ND-2000 spectrometer (Thermo, USA). The extracted DNA was stored at -80 °C for further sequencing and real-time PCR analysis. The prokaryote--specific primers 515F and 806R (GTGYCAGCMGCCGCGGTAA, GGACTACNVGGGTWTCTAA) (Kozich *et al*., 2013) were used to amplify the V4 region of 16S rRNA gene. To minimize reaction-level PCR bias, each sample was amplified in triplicate with the following reaction conditions: 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. Equimolar amounts of PCR products (assuming that amplicons of the same size had a similar molar mass) from each sample were combined in a single tube for high-throughput sequencing on an Illumina MiSeq platform (Illumina, San Diego, USA) in Novogene company (Beijing, China) which produced paired-ended reads.

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. The sequences with ambiguous bases or truncated at any site of more than three consecutive bases receiving a Phred quality score (Q) < 20 were deleted. Operation taxonomic units (OTUs) were clustered with 97% similarity by Usearch (vsesion 7.0) against the SILVA database (release128) (Edgar, 2013). Chimeric sequence and a single sequence without duplication were removed (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). The rarefaction curve was used to determine the sequencing depth by Mothur software. The Shannon, species richness and coverage indices were calculated for each sample using the methods of Mothur (Edgar *et al*., 2011). Community composition barplot was drawn using R script. Principal coordinates analysis (PCoA) was performed to evaluate the overall differences in bacterial community structure based on Bray-Curtis distance (Clarke *et al*., 1993). 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 to be significant at $p < 0.05$.

Real-time quantitative PCR

To determine the bacterial load in the coelomic fluid of sea cucumbers at different time post-evisceration, absolute quantitative PCR was conducted according to the method previously reported (Lu *et al*., 2017). 16S rRNA gene were amplified by rTaq DNA polymerase (Takara, Japan) using primers 27F/1492R (AGAGTTTGATCMTGGCTCAG, GGTTACCTTGTTACGACTT) (Goodfellow and Stackebrandt, 1991). 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 concentration of recombinant plasmid was measured by Nanodrop ND-1000 (Thermo, USA), and the copy number of standard plasmid was calculated according to the method described previously (Liu *et al*., 2016). The standard plasmid templates were diluted with a 1:10 gradient in DEPC water to the final copy numbers of 10^3 - 10^9. Real-time PCR were performed using the serially diluted templates with primers 341F/534R (CCTACGGGAGGCAGCAG, CGCGGCTGCTGGCACGTA) (Liu *et al*., 2011), and the threshold cycle (Ct) values against the denary logarithms of copy number were recorded and analyzed to establish a standard curve for copy number. Samples of genomic DNA were adjusted to a final concentration of 10 ng/μL for real-time quantitative PCR. After real-time quantitative PCR detection with the primers 341F/534R, the Ct value was recorded and analyzed. The total number of bacteria was calculated based on the standard curve and Ct value.

Results

The antibacterial activity of coelomic fluid at different post-evisceration times

The antibacterial activity of coelomic fluid against *V. splendidus* was evaluated by detecting

the bacteria growth curve. There was no significant difference of OD_{600} in the first nine hours among the coelomic fluid samples collected at different time points, but the values of OD_{600} for the coelomic fluid at 7dpe and 14 dpe were higher than that at 28 dpe from 10 h to 24 h (Fig. 1), indicating that the antibacterial abilities of coelomic fluid decreased firstly at 7 dpe and 14 dpe, and then recovered to the original level at 28 dpe.

The abundance of total bacteria in coelomic fluid at different time points post-evisceration

The abundance of total bacteria in different samples was examined by real-time quantitative PCR. The average copy number in Ctrl group was $5.125 \pm 0.76 \times 10^6$ copies/mL. After evisceration, the relatively lower copy numbers of bacterial loads were observed at 7 dpe and 14 dpe, which was about 50.7% ($2.6 \pm 0.5 \times 10^6$ copies/mL) and 72.3% ($3.74 \pm 0.89 \times 10^6$ copies/mL) of that in Ctrl group, respectively. At 28 dpe, the bacteria load was almost the same as the control group ($5.37 \pm 0.37 \times 10^6$ copies/mL) (Fig. 2).

The high-throughput sequencing data of V4 region in 16S rRNA gene

High-throughput sequencing of the V4 region in 16S rRNA gene was performed to determine bacterial diversity in coelomic fluid of *A. japonicus* at different time points post-evisceration. After the quality control of the raw sequences, a total of 901,496 clean tags with an average of 75,124 per sample were obtained. In the rarefaction curves, the number of OTUs almost reached the plateau phase with the increasing read number at 25,000, suggesting the sufficient sequencing depth in all samples (Fig. S1A). After rarefaction to equal sequencing depth, there were 465 OTUs across all samples (Fig. S1B). 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.

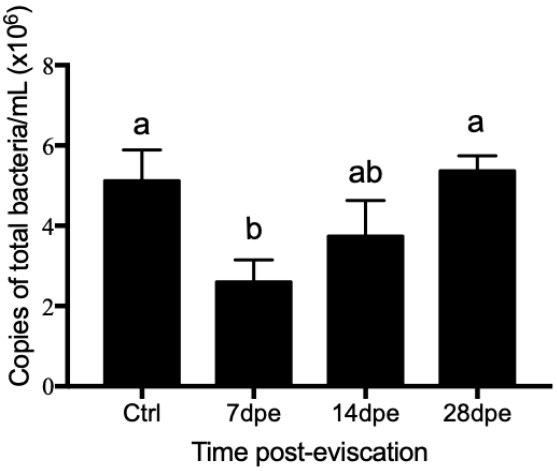

Fig. 2 The 16S rRNA gene copy number of the total bacteria by real-time quantitative PCR during different post-evisceration periods. Means ± standard deviations were compared using one-way analysis of variance (ANOVA). Different lowercase letters indicate significant differences ($p < 0.05$) among groups

Bacterial community dissimilarity among the coelomic fluid at the different time points post-evisceration

To estimate and compare the diversity of bacterial community among different samples, alpha- and beta-diversity of bacterial communities were determined. Alpha-diversity was estimated by the calculating Shannon index and Chao1 index. The Shannon index decreased significantly at 7 dpe (0.79-folds to the Ctrl group, $p < 0.05$), then increased slightly at 14 dpe (0.86-folds to the Ctrl group, $p > 0.05$), and returned to the level of Ctrl group at 28 dpe (0.92-folds to the Ctrl group, $p > 0.05$) (Fig. 3A). Chao1 index also declined obviously

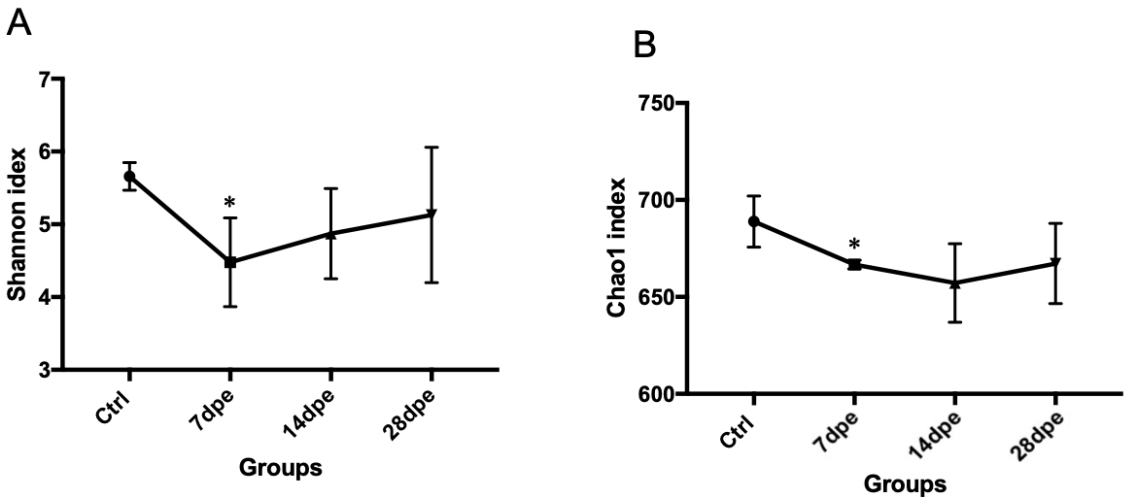

Fig. 3 Comparison of Shannon diversity (A) and Chao1 index (B) among different post-evisceration groups. Means ± standard deviations were compared using one-way analysis of variance (ANOVA). * indicates significant differences ($p < 0.05$) among groups

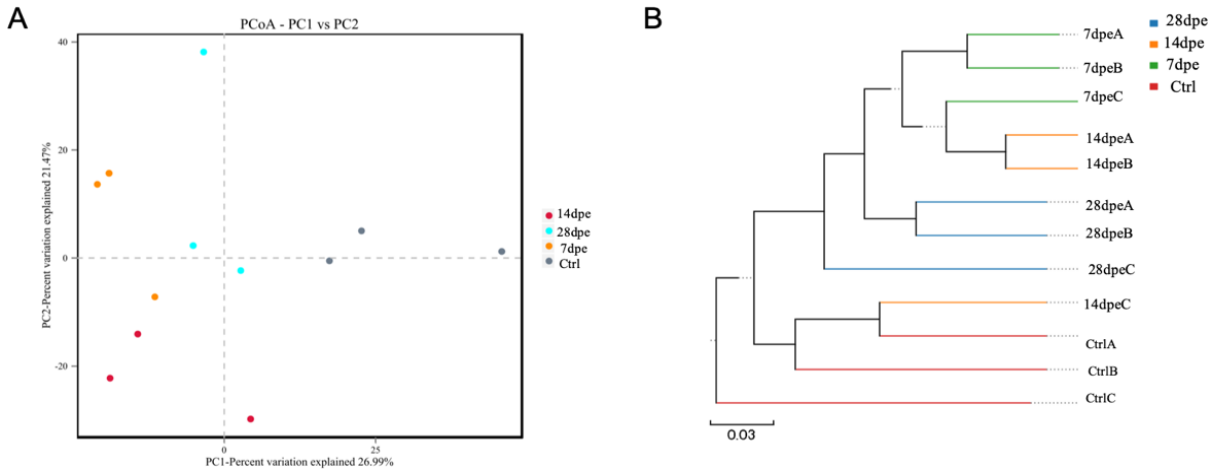

Fig. 4 Principal coordinates analysis (PCoA) of the dissimilarities of *A. japonicus* coelomic fluid bacterial community among the four groups based on Bray-Curtis distance. (B) Hierarchical clustering tree based on weighted UniFrac distances for sequences derived from *A. japonicus* for each of the replicate Ctrl, 7 dpe, 14 dpe and 28 dpe groups

at 7dpe ($p < 0.05$), but recovered to level of Ctrl group at 14 dpe and 28 dpe (Fig. 3B). The Beta-diversity of bacterial community among all the samples were explored by PCoA analysis (Fig. 4A). The bacterial community replicates were clustered separately in different group, and the bacterial community structure at 28 dpe was more closely resembled the control group. The community structure at 7 dpe was more different from that of the control group. These patterns were further corroborated by the hierarchical clustering tree among groups (Fig. 4B).

Taxonomic distribution and phylotypes of coelomic fluid bacterial community at different time points post-evisceration

In the present study, 97.7 % of the sequences were classified into 10 phyla. In the Ctrl group, the dominant phyla in coelomic fluid were Proteobacteria (69.37 ± 1.02 %), followed by Firmicutes (10.47 ± 2.15 %), Verrucomicrobia (8.47 ± 1.29 %), Bacteroidetes and Actinobacteria (4.47 ± 1.75 %) (Fig. 5A). The taxonomic distribution of coelomic fluid bacterial was companied large change at the phylum level in the Ctrl and different post-evisceration periods. The relative abundance of Bacteroidetes reached the highest level at 7 dpe (3.2-fold higher than that in Ctrl group, $p < 0.01$), and then went down at 28 dpe, which was 1.6-fold higher ($p > 0.05$) than that in Ctrl group (Fig. 5B). In addition, the ratio of Bacteroidetes and Firmicutes increased significantly at 7 dpe and 14 dpe, which was 17.2-folds ($p < 0.01$) and 10.4-folds ($p < 0.05$) compared with Ctrl, respectively (Fig. 5C), and then decreased at 28 dpe ($p > 0.05$).

Significant changes of coelomic fluid bacterial assemblages at the family level after evisceration

The bacterial community at family level generates the highest ecological potential to indicate host health status (Xiong *et al*., 2014). In the Ctrl group, the dominant family in coelomic fluid was Colwelliaceae (21.37 ± 2.14 %), followed by Rhodobacteraceae (11.47 ± 3.17 %), Enterobacteriaceae (6.47 ± 1.09%), Rubritaleaceae (5.42 ± 1.15 %) and Vibrionaceae (3.17 ± 1.78 %) (Fig. 6A). At 7 dpe, the dominant family in coelomic fluid was Rubritaleaceae (17.05 ± 3.15 %), followed by Hyphomonadaceae (10.47 ± 3.67 %), Enterobacteriaceae (6.47 ± 2.13 %), Rhodobacteraceae (5.40 ± 1.18 %) and Vibrionaceae (4.17 ± 2.05 %) (Fig. 6A). Enterobacteriaceae was found to be the dominant family at 14 dpe (20.27 ± 5.16 %) in coelomic fluid, followed by Rhodobacteraceae (10.20 ± 2.90 %), Rubritaleaceae (8.91 ± 4.15 %), Vibrionaceae (6.80 ± 4.10 %) and Hyphomonadaceae (4.87 ± 1.24 %) (Fig. 6A). At 28 dpe, the most abundant family in coelomic fluid was Rhodobacteraceae (25.30 ± 5.12 %), followed by Enterobacteriaceae (9.74 ± 1.17 %), Rubritaleaceae (5.07 ± 1.29%), Colwelliaceae (2.32 ± 1.12 %) and Vibrionaceae (1.57 ± 1.71 %) (Fig. 6A). Specifically, the abundance of Rhodobacterales also increased significantly from 14 dpe to 28 dpe (Fig. 6A). While the relative abundance of Rubritaleaceae increased significantly at 7 dpe (3.42-fold compared with Ctrl, $p < 0.01$), then dropped to the similar level as Ctrl group at 28 dpe (Fig. 6B).

Prediction of bacterial community functions at different post-evisceration periods

Due to the lowest bacterial community diversity and maximum bacterial community structural change at 7 dpe, the functions the bacterial communities before evisceration as well as those at 7 dpe and 28 dpe were predicted by PICRUSt. (Langille *et al.*, 2013). The metabolism related pathways were around the top-rated enriched pathways. The abundances of amino acid metabolism, lipid metabolism and carbohydrate

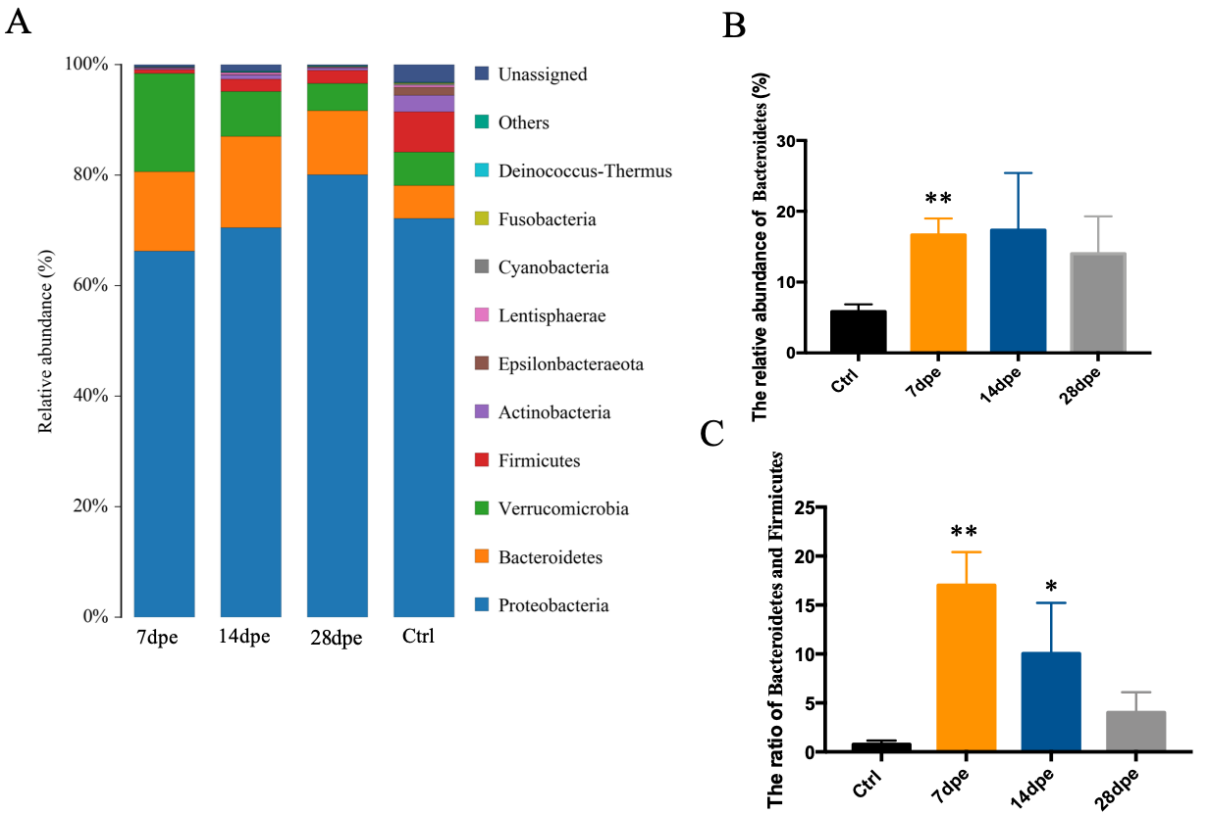

Fig. 5 Comparison of the relative abundances of major bacterial phyla. (A) Histogram of species distribution. (B) Relative abundance of Bacteroidetes in different post-evisceration groups. (C) The ratio of Bacteroidetes and Firmicutes in different post-evisceration groups. Means ± standard deviations were compared using one-way analysis of variance (ANOVA). ** indicates significant differences ($p < 0.01$) among groups. * indicate significant differences ($p < 0.01$) among groups

metabolism pathways all increased significantly at 7 dpe compared with Ctrl group (Fig. 7A). Compared with that at 7 dpe, the abundance of amino acid metabolism pathway was upregulated while the abundance of carbohydrate metabolism pathway was downregulated, and lipid metabolism pathway showed no significant change at 28 dpe (Fig. 7B). The nucleotide metabolism pathway decreased at both 7 dpe and 28 dpe (Fig. 7A, B).

Discussion

Sea cucumber is considered as a good organism for studying organ regeneration and organogenesis for its ability to regenerate self-eviscerated organs within a few weeks (Ortiz-Pineda *et al*., 2009). Microbiota exerts either positive or negative influences on the health status of aquatic animals, by symbiotic relationship or causing diseases, respectively (Antunes *et al*., 2010). Increasing evidences indicate the important roles of microbiota in organ regeneration of animals (Zhang *et al*., 2020; Stedman *et al*., 2016; Khosravi *et al*., 2014; Arnold *et al*., 2016; Zhang *et al*., 2019; Wang *et al*., 2018). However, the information about the dynamics of coelomic fluid microbiota after evisceration is very limited. In the present study, the bacterial community in coelomic fluid of *A. japonicus* after evisceration was investigated, and a significant variation was observed during early stage after evisceration.

The interaction between microbial community and host is the main factor to maintain the halobiont homeostasis. Echinodermata coelomic fluid contains abundant antimicrobial compounds (Dybas *et al*., 1986), which can directly influence the growth of microbes in coelomic fluid. Although the regeneration process of coelomocytes has been well described (Li *et al*., 2018)，little was known about the variation of the antimicrobial ability during the process. In the present study, the antibacterial ability of the coelomic fluid was found to decrease firstly at 7 dpe and 14 dpe, and then recovered at 28 dpe. For the bacterial community, the total bacteria load was slightly increased from 7 dpe to 14 dpe and recovered to the original at 28 dpe. These results indicated that the lower antibacterial ability of coelomic fluid at 7 dpe and 14 dpe provided an ideal hotbed for the bacterial population. While the species richness (Chao1 index) and diversity (Shannon index) of coelomic fluid bacteria were significantly lower at 7 dpe. Previous studies revealed a sharp decline in the species richness and diversity of intestinal bacteria in the early stage

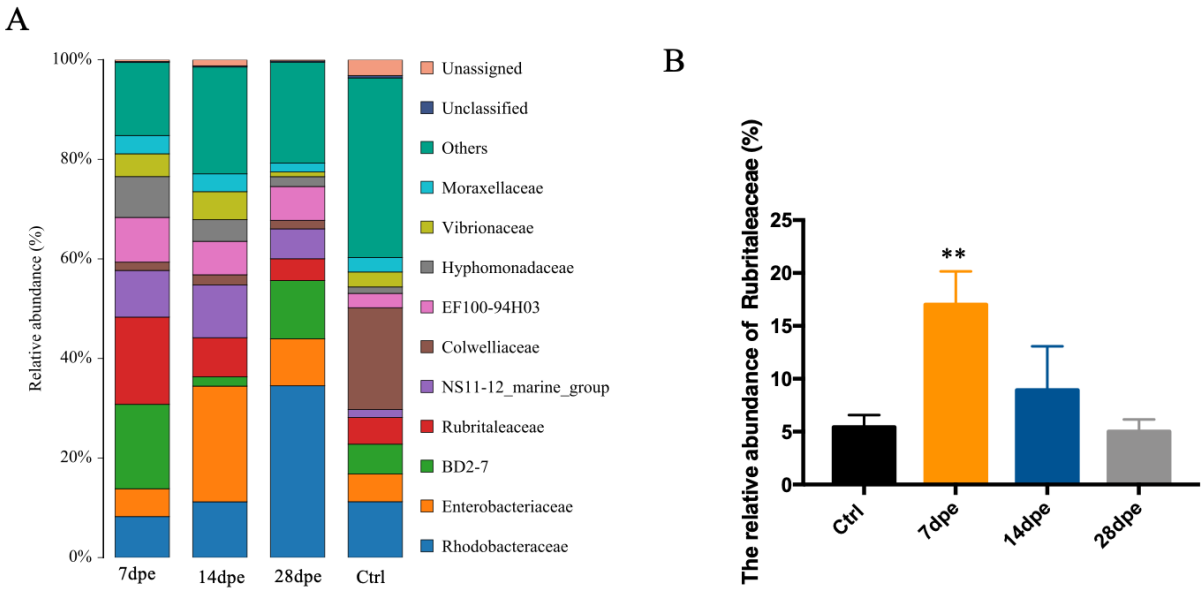

Fig. 6 Comparison of the relative abundances of major bacterial family. (A) Histogram of species distribution. (B) Relative abundance of Rubritaleaceae in different post-evisceration groups. ** indicates significant differences ($p < 0.01$) among groups

of intestinal regeneration, and the diversity of intestinal bacteria then increased gradually (Zhang *et al*., 2019). Ecological evidence indicated that reduced bacterial amount and diversity would provide vacant niches for invading pathogens (Mallon *et al*., 2015). In the current study, the reduced bacterial diversity and total bacteria load at 7 dpe might indicate the decrease of functional stability of bacterial community, which would increase the risk of developing diseases (Xiong *et al*., 2017). And the higher antibacterial ability at 7 dpe (compared with that at 14 dpe) might contribute to inhibiting the thriving of harmful bacterial species at the early stage after evisceration.

The dynamics of bacterial community after evisceration was determined by beta-diversity analysis. Both PCoA and hierarchical clustering analyses indicated that the bacterial community structure at 7 dpe was significantly different from the normal level, and it began to restore at 28 dpe. The results were consistent with previous report on the bacterial community in intestine of *A. japonicus* that the intestinal bacterial community varied significantly and tended to be stable when the regeneration was accomplished (Zhang *et al*., 2019).

In the present study, the bacterial community in *A. japonicus* coelomic fluid was found to be dominated by Proteobacteria, following by Firmicutes, Bacteroidetes and Verrucomicrobia, which was consistent with the previous report (Enomoto *et al*., 2012). Interestingly, Bacteroidetes increased significantly at 7 dpe and 14 dpe, and returned to pre-evisceration level at 28 dpe. Previous study showed that the relative abundance of Bacteroidetes in *A. japonicus* intestinal also increased in the middle stage of regeneration and returned to pre-evisceration levels in the end stage (Zhang *et al*., 2019). Bacteroidetes can ferment polysaccharides and indigestible carbohydrates to produce short-chain fatty acids (SCFAs) that beneficial for the host (Faintuch and Faintuch, 2019). The present results suggested that the bacteria in coelomic fluid might help to provide energy for the host before the intestine was fully grown. In addition, the ratio of Bacteroidetes and Firmicutes was found to increase significantly at 7 dpe and 14 dpe. It has been reported that that the ratio reduction of Bacteroidetes and Firmicutes in mouse intestine promoted fat storage (Backhed *et al*., 2004). Our result on the contrary indicated that the bacteria might promote the lipolysis, which helped to provide the energy. Rubritaleaceae, confirmed to contribute to polysaccharide degradation (Martinez-Garcia *et al*., 2012), was also found to increase significantly at 7 dpe. Previous study showed the abundance of Rhodobacterales in intestinal increased significantly in samples from 14 dpe to 21 dpe in the intestinal regeneration process, and it was predicted to function as keystone taxa in intestinal community (Zhang *et al*., 2019). In deriving germ-free *A. japonicus,* the abundance of Rhodobacteraceae increased to promote the intestine regrowth rate of *A. japonicus* (Zhang *et al*., 2020). In the present study, the abundance of Rhodobacterales in coelomic fluid was also found to increase significantly from 14 dpe to 28 dpe. As Rhodobacterales retains polyhydroxybutyrate (PHB) metabolism genes to promote the growth of *A. japonicus* (Yamazaki *et al*., 2016), it is also hypothesized to be a keystone taxa in coelomic fluid after evisceration. It was generally believed that the disruption of bacteria homeostasis can alter bacterial-mediated functions, which in turn affects the growth of animals (Xiong *et al*., 2017). The synchronic changes in microbial composition

Fig. 7 Comparison of *A. japonicus* coelomic fluid bacterial KEGG pathways between Ctrl with 7 dpe (days post–evisceration) groups (A), and 7 dpe with 28 dpe groups (B) using the response ratio methods

and function have been well demonstrated, especially the metabolic pathways of three major nutrients (Catalan *et al*., 2018). In the present study, the pathways related to amino acid metabolism, lipid metabolism and carbohydrate metabolism were found to be more abundant in 7 dpe. Intestine is the main digestive organ, but it has no digestive function until its regeneration is complete. All these results suggested that the coelomic fluid bacteria might decompose polysaccharides and lipid to provide energy after evisceration.

In summary, the temporal dynamics of coelomic fluid bacterial community in *A. japonicus* was investigated after evisceration. The bacterial community structure changed greatly after evisceration and it gradually recovered and tended to be stable at 28 dpe. The coelomic fluid bacterial community was involved in the decomposition of polysaccharides and lipid before the recovery of intestinal digestive function from evisceration.

Acknowledgments

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