J. Ocean Univ. China (Oceanic and Coastal Sea Research)
DOI 10.1007/s11802-016-3092-8
ISSN 1672-5182, 2016 15 (6): 1067-1072
http://www.ouc.edu.cn/xbywb/
E-mail:xbywb@ouc.edu.cn

Characterization of Bacterial Communities Associating with Larval Development of Yesso Scallop (*Patinopecten yessoensisis* Jay, 1857) by High-Throughput Sequencing

SUN Xueying[1)], LIU Jichen[1)], LI Ming[2)], ZHAO Xuewei[2)], LIANG Jun[2)], SUN Pihai[3)], and MA Yuexin[1), *]

1) *Key Laboratory of Mariculture & Stock Enhancement in North China's Sea of Ministry of Agriculture*, *Dalian Ocean University*, *Dalian* 116023, *P. R. China*
2) *Zhangzi Island Group Co*., *Ltd.*, *Dalian* 116001, *P. R. China*
3) *Seafood Seedling Breeding Base*, *Dalian Ocean University*, *Dalian* 116023, *P. R. China*

(Received December 23, 2015; revised April 23, 2016; accepted September 29, 2016)

Abstract Bacterial community presumably plays an essential role in inhibiting pathogen colonization and maintaining the health of scallop larvae, but limiting data are available for Yesso scallop (*Patinopecten yessoensisis* Jay, 1857) larval development stages. The aim of this study was to characterize and compare the bacterial communities associating with Yesso scallop larval development at fertilized egg S1, trochophora S2, D-shaped larvae S3, umbo larvae S4, and juvenile scallop S5 stages by Illumina high-throughput sequencing. Genomic DNA was extracted from the larvae and their associating bactera, and a gene segment covering V3-V4 region of 16S rRNA gene was amplified and sequenced using an Illumina Miseq sequencer. Overall, 106760 qualified sequences with an average length of 449 bp were obtained. Sequences were compared with those retrieved from 16S rRNA gene databases, and 4 phyla, 7 classes, 15 orders, 21 families, 31 genera were identified. Proteobacteria was predominant phylum, accounting for more than 99%, at all 5 larval development stages. At genus level, *Pseudomonas* was dominant at stages S1 (80.60%), S2 (87.77%) and S5 (68.71%), followed by *Photobacterium* (17.06%) and *Aeromonas* (1.64%) at stage S1, *Serratia* (6.94%), *Stenotrophomonas* (3.08%) and *Acinetobacter* (1.2%) at stage S2, *Shewanella* (25.95%) and *Pseudoalteromonas* (4.57%) at stage S5. Moreover, genus *Pseudoalteromonas* became dominant at stages S3 (44.85%) and S4 (56.02%), followed by *Photobacterium* (29.82%), *Pseudomonas* (11.86%), *Aliivibrio* (8.60%) and *Shewanella* (3.39%) at stage S3, *Pseudomonas* (18.16%), *Aliivibrio* (14.29%), *Shewanella* (4.11%), *Psychromonas* (4.04%) and *Psychrobacter* (1.81%) at stage S4. From the results, we concluded that the bacterial community changed significantly at different development stages of Yesso Scallop larvae.

Key words *Patinopecten yessoensisis*; larval development stage; bacterial community; high-throughput sequencing

1 Introduction

The commercial scale cultivation of Yesso scallop (*Patinopecten yessoensis*) in Liaoning and Shandong Provinces, China, makes an important contribution to the socioeconomic development of these coastal regions. Nonetheless, mass mortality occurred frequently during the seed production due to opportunistic pathogens, which has become one of the major limiting factors for the culture of Yesso scallop (Liu *et al*., 2015). The serious economic loss associated with hatchery failures is apparently due to bacterial infections, which has promoted researches into the presence of bacteria at various steps of seed production. Studies are appreciated to define the problem and produce applicable biotechnology which may aid to producing reliable scallop seeds by the commercial growers, thus supplying the seeds year-round.

Like other bivalves, scallop larvae are filter-feeding, and are exposed to large quantities of bacteria. Bacteria play crucial roles in causing larval diseases. The study on bacterial community is the first step to manipulate a bacterial population with an aim of identifying possible pathogens and probiotics. The knowledge of bacterial community associated with larvae will be essential for improving the seed production. Bacterial diversity and community structure associated with scallop and oyster larvae have been studied previously using culture-dependent technique (Godoy *et al*., 2011), denaturing gradient gel electrophoresis (DGGE) (Sandaa *et al*., 2003), temperature gradient gel electrophoresis (TGGE) and restriction fragment length polymorphism (RFLP) (Trabal *et al*., 2012).

Massively parallel next generation sequencing (NGS) platforms (Shendure *et al*., 2008) have now been used for

* Corresponding author. Tel: 0086-411-84763096
E-mail: mayuexin_1963@163.com

a broad range of applications to address diverse biological problems (Liang *et al*., 2011). The bacterial and archaeal diversities have been investigated on NGS platforms in the marine environment (Angly *et al*., 2006; Huber *et al*., 2007; Desnues *et al*., 2008; Galand *et al*., 2009; Gilbert *et al*., 2010; Gilbert *et al*., 2012). Recently, the microbiota associating with reared Great scallop (*Pecten maximus*) gonads before and after spawning (Lasa *et al*., 2014) and the bacterial diversity in the mantle of healthy and incised symptoms of Yesso scallop (Ding *et al*., 2014) have also been studied by pyrosequencing 16S rRNA gene. However, less is known about the bacterial composition associated with Yesso scallop larvae. The aim of this study was to characterize and compare bacterial communities associating Yesso scallop larval development at different stages by Illumina high-throughput sequencing, thus providing the basis for managing scallop seedling raising systems.

2 Materials and Methods

2.1 Sample Collection

Yesso scallop larvae were reared in seawater in 100 L tanks under static conditions at a density of 5–6 larvae mL^{-1} and fed *Dicrateria inornata*, 100 mL, 7–9×10^5 cells mL^{-1}, three times a day. Low-pressure electrical blowers provided aeration via air stones. Water temperature was kept at 15±1℃ and salinity at 32±0.5 (Practical Salinity Units). Half (50%) of the seawater each tank was replaced every day. Mortality occurred from D-shaped larvae to umbo larvae. The sick larvae sunk to the bottom and the healthy larvae suspended in the seawater. Healthy larvae were collected from three tanks with gauze during development lasting for five stages. Then three larvae samples at each stage were collected and pooled equally. The mixture of larvae (approximately 0.1 g) were rinsed three times with sterilized seawater to remove non-attached bacteria, and then homogenized fully with 0.4 mL sterilized seawater. The homogenates were immediately stored at −80℃ until DNA extraction.

2.2 DNA Extraction and PCR Amplification

Genomic DNA was extracted using a Rapid Soil DNA Isolation Kit (Shanghai Sangon Biological Engineering Technology and Services Company Limited, Shanghai, China) according to manufacturer's instructions. A gene segment (V3-V4 portion) of 16S rRNA gene was amplified through PCR by predenaturing at 95℃ for 2 min followed by 30 cycles of denaturing at 95℃ for 30 s, annealing at 55℃ for 30 s and extending at 72℃ for 45 s, and an additional extension cycle at 72℃ for 10 min. The gene was amplified with primers 338F (5'-barcode-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Lane, 1991; McBain *et al*., 2003), in which barcode is an eight-base sequence unique to each sample. PCR was performed in triplicate and the reaction volume was 20 μL containing 4 μL of 5×FastPfu buffer, 2 μL of 2.5 mmol L^{-1} dNTP (each nucleotide), 0.4 μL of 5 μmol L^{-1} primers (each direction), 0.4 μL of FastPfu polymerase (2.5 U μL^{-1}), and 10 ng of template DNA.

2.3 Illumina MiSeq Sequencing

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, U.S.). Purified amplicons were pooled in equimolar and paired-end sequenced (2×300) on an Illumina MiSeq platform following standard protocols (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China).

2.4 Processing of Sequencing Data

Raw fastq files were demultiplexed, and quality-filtered using QIIME (version 1.17) (Caporaso *et al*., 2010) with the following criteria: (i) 300 bp reads truncated at any site over a 50 bp sliding window scored in average <20, truncated reads shorter than 50 bp; (ii) any mismatched in barcode region, 2 nucleotide mismatched in primer and reads containing ambiguous characters (iii) only sequences that overlap longer than 10 bp assembled and reads failed to be assembled discarded. Operational Taxonomy Units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) (Edgar *et al*., 2011) and chimeric sequences were identified and removed using UCHIME (Edgar 2013). The most abundant sequence each OTU was chosen as the representative. The taxonomy each 16S rRNA gene sequence was analyzed by RDP Classifier (http: //rdp.cme.msu.edu/) (Wang *et al*., 2007) against the silva (SSU115) 16S rRNA database (Quast *et al*., 2013) using confidence threshold of 70% (Amato *et al*., 2013). Rarefaction analysis, Coverage (the Good's coverage) (http://www.mothur.org/wiki/Coverage), Ace (the Ace estimator) (http://www.mothur.org/wiki/Ace), Chao (the Chao1 estimator) (http://www.mothur.org/wiki/Chao), Shannon index (http://www.mothur.org/wiki/Shannon) and Simpson index (http://www.mothur.org/wiki/Simpson) were created using mothur software (version v.1.30.1 http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity). Coverage percentage was calculated as $[1–(n/N)]\times100$, where n represents the number of single-member phylotypes and N represents the number of sequences. Venn, mcrobial community barplot, and Heatmap were performed in R software.

3 Results

3.1 Illumina Miseq Sequencing Data

In total, 106760 valid sequences with an average length of 449 bp were obtained from larvae samples. The number of sequences of each larvae sample was showed in Table 1. Rarefaction curves generated at the OTUs level were approached to saturation (Fig.1), indicating that the sequencing depth was enough to reflect the diversity. Sixty five OTUs were obtained from larvae samples. The number of OTUs of each larvae sample ranged from 29 to

38 (Table 1, Fig.2). Our analysis revealed 4 phyla, 7 classes, 15 orders, 21 families and 31 genera in total.

Table 1 Alpha diversity obtained at different development stages of Yesso scallop larvae

Sample	Reads	OTU	Coverage (%)	Ace	Chao	Shannon	Simpson
S1	25842	37	99.95	50	48	0.63	0.67
S2	15174	34	99.91	51	49	1.27	0.40
S3	14685	38	99.93	50	46	1.78	0.24
S4	19937	29	99.98	31	31	1.47	0.34
S5	15719	31	99.94	42	42	1.51	0.30

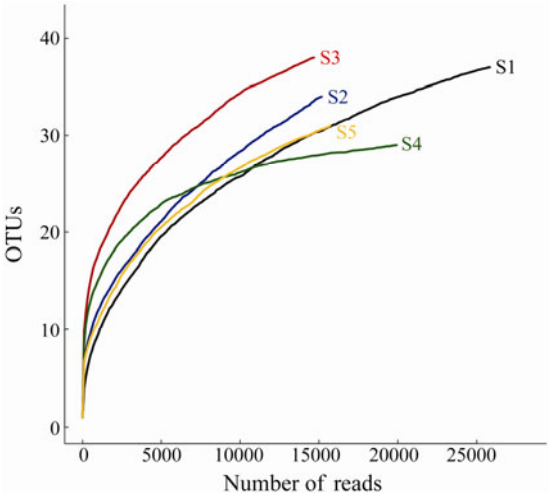

Fig.1 Rarefaction curves of OTUs clustered at 97% similarity among development stages of Yesso scallop larvae.

3.2 Species Richness and Diversity Estimates

Bacterial taxonomic richness and diversity varied greatly among stages. As indicated by Chao and Ace indices, stage S2 harbored the highest community richness, followed by stages S1, S3 and S5, while the lowest community richness was found at stage S4. Simpson and Shannon indices showed that the bacterial diversity increased in order of stages S1, S2, S4, S5, and S3.

3.3 Community Composition

The shared OTUs were shown *via* Venn diagram (Fig.2). Each stage contained only a minority of special OTUs (varied between 1 and 9). Most of OTUs (16–26) were shared between stages.

A total of 4 phyla, Proteobacteria, Bacteroidetes, Firmicutes and Fusobacteria, were identified among 5 stages, of them Proteobacteria covered richest 16S rRNA gene sequences (>99%) (Table 2) while the other 3 stages covered < 1% of the total. Sequences representing Proteobacteria and Bacteroidetes were found at all stages exception S1 and S4 which contained 100% of Proteobacteria. Sequences from Firmicutes were only detected at S3 and those from Fusobacteria were detected only at S5.

Candidates division was observed at genus level (Fig.3), and 31 genera were identified in total. Genera 22, 22, 20, 16 and 21 were obtained at S1 through S5, respectively. The most abundant 16S rDNA sequences at S1 (80.60%), S2 (87.77%) and S5 (68.71%) belonged to *Pseudomonas*; followed by those to *Photobacterium* (17.06%) and *Aeromonas* (1.64%) at S1; *Serratia* (6.94%), *Stenotrophomonas* (3.08%) and *Acinetobacter* (1.21%) at S2; *Shewanella* (25.95%) and *Pseudoalteromonas* (4.57%) at S5. However, reads assigned to *Pseudoalteromonas* became the dominant at S3 (44.85%) and S4 (56.02%); followed by those to *Photobacterium* (29.82%), *Pseudomonas* (11.86%), *Aliivibrio* (8.60%) and *Shewanella* (3.39%) at S3; *Pseudomonas* (18.16%), *Aliivibrio* (14.29%), *Shewanella* (4.11%), *Psychromonas* (4.04%) and *Psychrobacter* (1.81%) at S4. The percentage of other genera at 5 stages was less than 1%.

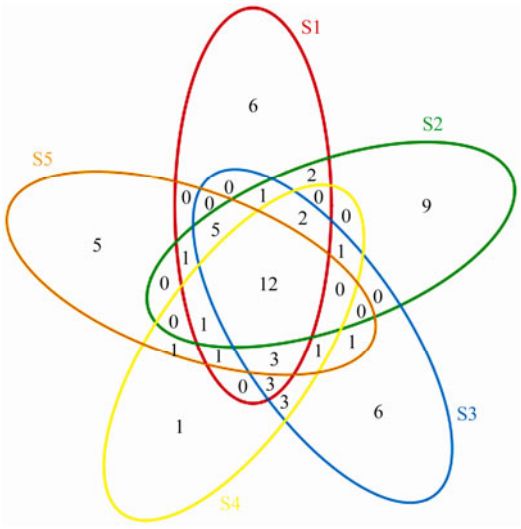

Fig.2 Venn diagram showing OTUs unique to and shared by Yesso scallop larval development stages.

Table 2 Phylum abundance (%) at Yesso scallop larval development stages

Stage	Proteobacteria	Bacteroidetes	Firmicutes	Fusobacteria
S1	100	0	0	0
S2	99.70	0.30	0	0
S3	99.96	0.01	0.03	0
S4	100	0	0	0
S5	99.92	0.04	0	0.04

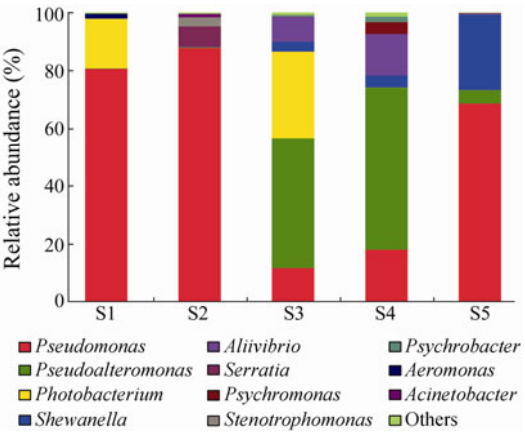

Fig.3 Genus abundance (%) at Yesso scallop larval development stages. Sequence abundance less than 1% at a stage was combined into ‘others’.

The genus abundance distribution among 5 stages is shown in Fig.4. All 5 stages formed 2 clades, of them S1 and S2 merged first then with S5, and S3 and S4 merged as the other clade.

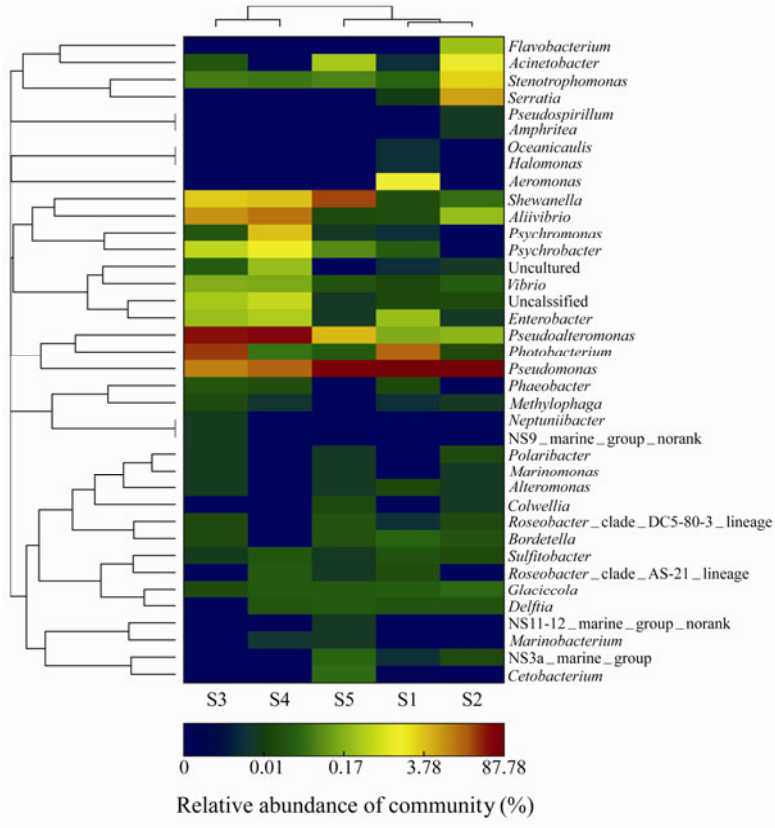

Fig.4 Heatmap based on the genus abundance similarity. Double hierarchical dendrogram shows the bacterial distribution among 5 stages. The heatmap plot depicts the relative percentage each genus (rows) at each stage (columns). The relative abundance of bacterial genera is depicted by color intensity with the legend appeared at the bottom of the figure. Clusters based on the distance of 5 stages along *X*-axis and the bacterial genera along *Y*-axis are indicated in the upper and left of the figure, respectively.

4 Discussion

In this study, a well-defined trend in bacterial community succession was observed during development of scallop larvae (Figs.3 and 4). Such trend was in agreement with the described previously in Chilean scallop (*Argopecten purpuratus*). When the larval culture progressed, the predominance and composition of bacterial genera changed (Godoy *et al*., 2011). Similarly, the bacterial community composition as revealed by DGGE profiling varied during the growth of Great scallop larvae (Sandaa *et al*., 2003). Proteobacteria was the predominant phylum, accounting for more than 99% at 5 stages of Yesso scallop larval development. This phylum was also the dominant around healthy adult Yesso scallop (adductor muscle, foot, gill, gonad, haemolymph, intestine and mantle) (Schulze *et al*. 2006; Ding *et al*. 2014). Similar result was found by Godoy *et al.* (2011) in Chilean scallop larvae in which Proteobacteria accounted for 65% of the total. At the genus level, when data from the 5 larval development stages were considered together, *Pseudomonas*, *Pseudoalteromonas*, *Photobacterium*, *Shewanella*, *Aliivibrio*, *Serratia*, *Psychromonas*, *Stenotrophomonas*, *Psychrobacter*, *Aeromonas* and *Acinetobacter* (>1%) were the top 11 genera. All of them have been previously reported as part of the bivalve larvae or adult bacterial community, for example, *Pseudomonas*, *Pseudoalteromonas*, *Photobacterium* and *Shewanella* were detected with culture-dependent method and DGGE in healthy adult Yesso scallop (Schuze *et al*., 2006). Culturable *Pseudomonas* and *Pseudoalteromonas* were found to be dominant at larval stage of Chilean scallop (Riquelme *et al*., 1994; Godoy *et al*., 2011). *Pseudoalteromonas*-like bacteria were confirmed to be associated with larvae of Great scallop through DGGE (Sandaa *et al*., 2003). *Acinetobacter* and *Pseudomonas* were among the most prevalent genera associating with reared Great scallop gonads before and after spawning as was demonstrated by pyrosequencing 16S rRNA gene (Lasa *et al*., 2014). *Pseudomonas*, *Pseudoalteromonas*, *Photobacterium*, *Shewanella*, *Stenotrophomonas*, *Aeromonas* and *Acinetobacter* were found in wild adult Pacific oyster *Crassostrea gigas* (Kueh and Chan, 1985; Schuze *et al.*, 2006; Gomez-Gil *et al*., 2011). *Pseudomonas*, *Aeromonas* and *Acinetobacter* could be isolated from the sea mussel *Perna uiridis* and the arkshell clam *Scapharca cornea* (Kueh and Chan, 1985). *Pseudoalteromonas*, *Photobacterium*, *Shewanella*, *Serratia*, *Stenotrophomonas*, *Aliivibrio*, *Psychromonas*,

Psychrobacter and *Aeromonas* could be detected in Manila clams (*Ruditapes philippinarum*) or the carpet-shell clam *Ruditapes decussatus* (Schuze *et al*., 2006; Bourouni *et al*., 2007; Hidalgo *et al*., 2008; Romalde *et al*., 2013).

In this study, larval mortality occurred during the transition from the D-shaped to umbo larvae and at the entire period of stage S4. This coincided with the great change of bacterial community which significantly differs at S1, S2 and S5 (Figs.3 and 4). Relative abundance of *Pseudoalteromonas*, *Aliivibrio*, *Psychromonas*, *Psychrobacter* and *Vibrio* at S3 and S4 were higher than those at other 3 stages (Figs.3 and 4). *Vibrio splendidus*- and *V. tasmaniensis*-like strains have been found to be associated with mass mortality of Yesso scallop larvae in hatchery of Zhang Zidao Island Group (Liu *et al.*, 2015). The sequences of OTU 102 (*V. splendidus*) at stages S3 and S4 was more abundant than that at other 3 stages. *V. splendidus* has also been identified as the pathogen for the mass mortality of Great scallop larvae in a hatchery near Bergen, western Norway (Torkildsen *et al*., 2005). A *Vibrio anguillarum*-related strain that is pathogenic to Chilean scallop larvae has been identified (Riquelme *et al*., 1995), while *Vibrio pectenicida* has been isolated and described by Lambert *et al*. (1998) as the major cause of mortality during the production of Great scallop larvae in France. *Pseudoalteromonas* sp. LT13 produced high mortality in the challenge test in 10- to 16-day-old Great scallop larvae (Torkildsen *et al*., 2005). For the bacteria in the present study, an assessment of the virulences of species in genera *Pseudoalteromonas*, *Aliivibrio*, *Psychromonas* and *Psychrobacter* in the Yesso scallop larvae is warranted.

Previous studies demonstrated that bacteria may benefit scallop larvae. *Pseudoalteromonas haloplanktis* (formerly *Alteromonas haloplanktis*) and *Vibrio* sp. were able to confer protection to Chilean scallop larvae against infection of pathogenic *Vibrio anguillarum* (Riquelme *et al*., 1996; Riquelme *et al*., 1997). Riquelme *et al*. (2001) carried-out experiments by adding the inhibitory-producer bacterial strains to massive *A. purpuratus* larval cultures. The inoculation of mixtures of strains *Vibrio* sp. C33 and *Pseudomonas* sp. 11 made the development of larval stages without antibiotics possible. In the culture of Great scallop larvae, protection against *Vibrio coralliilyticus* and *V. splendidus* was provided by prior administration of *Phaeobacter allaeciensis* and *Alteromonas macleodii* 0444. Significant protection with *Pseudoalteromonas* sp. D41 was only provided against *V. splendidus* (Kesarcodi-Watson *et al*., 2012). However, whether the bacteria in genera of *Pseudoalteromonas*, *Vibrio*, *Pseudomonas*, *Phaeobacter* and *Alteromonas* found in this study have a probiotic effect in Yesso scallop larvae culture is unclear and requires further investigation.

In conclusions, a clear trend in bacterial community succession was demonstrated across different Yesso scallop larval development stages. With our knowledge, this is the first detailed study of Yesso scallop larvae-associated bacterial communities by high-throughput sequencing. It extended current knowledge on microbial community dynamics in a scallop larval development.

Acknowledgement

We acknowledge the financial support from Zhang Zidao Sland Group Co., Ltd. for the project (99801214).

References

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Gaskins, H. R., Stumpf, R. M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B. A., Nelson, K. E., White, B. A., and Leigh, S. R., 2013. Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *The ISME Journal*, **7**: 1344-5313.

Angly, F. E., Felts, B., Breitbart, M., Salamon, P., Edwards, R. A., Carlson, C., Chan, A. M., Haynes, M., Kelley, S., Liu, H., Mahaffy, J. M., Mueller, J. E., Nulton, J., Olson, R., Parsons, R., Rayhawk, S., Suttle, C. A., and Rohwer, F., 2006. The marine viromes of four oceanic regions. *PLOS Biology*, **4**: e368.

Bourouni, O. C., Bour, M. E., Mraouna, R., Abdennaceur, H., and Boudabous, A., 2007. Preliminary selection study of potential probiotic bacteria from aquacultural area in tunisia. *Annals of Microbiology*, **57**: 185-190.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, **7**: 335-336.

Desnues, C., Rodriguez-Brito, B., Rayhawk, S., Kelley, S., Tran, T., Haynes, M., Liu, H., Furlan, M., Wegley, L., Chau, B., Ruan, Y. J., Hall, D., Angly, F. E., Edwards, R. A., Li, L. L., Thurber, R. V., Reid, R. P., Siefert, J., Souza, V., Valentine, D. L., Swan, B. K., Breitbart, M., and Rohwer, F., 2008. Biodiversity and biogeography of phages in modern stromalites and thrombolites. *Nature*, **452**: 340-345.

Ding, J., Dou, Y., Xu, G. R., Wang, Y. N., and Chang, Y. Q., 2014. Bacterial diversity in the mantle of *Patinopecten yessoensis* revealed by 454 pyrosequencing. *Chinese Journal of Applied Ecology*, **25**: 3344-3328 (in Chinese with English abstract).

Edgar, R. C., 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, **10**: 996-998.

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.

Galand, P. E., Casamayor, E. O., KirchmanD. L., and Lovejoy, C., 2009. Ecology of the rare microbial biosphere of the Arctic Ocean. *Proceedings of the National Academy of Sciences of the United States of America*, **106**: 22427-22432.

Gilbert, J. A, Field, D., Swift, P., Thomas, S., Cummings, D., Temperton, B., Weynberg, K., Huse, S., Hughes, M., Joint, I., Somerfield, P. J., and Mühling, M., 2010. The taxonomic and functional diversity of microbes at a temperate coastal site: A 'multi-omic' study of seasonal and diel temporal variation. *PLoS One*, **5**: e15545.

Gilbert, J. A., Steele, J. A., Caporaso, J. G., Steinbrück, L.,

Reeder, J., Temperton, B., Huse, S., McHardy, A. C., Knight, R., Joint, I., Somerfield, P., Fuhrman, J. A., and Field, D., 2012. Defining seasonal marine microbial community dynamics. *ISME Journal*, **6**: 298-308.

Godoy, F. A., Espinoza, M., Wittwer, G., Uriarte, I., and Aranda, C., 2011. Characterization of culturable bacteria in larval cultures of the Chilean scallop *Argopecten purpuratus*. *Ciencias Marinas*, **37**: 339-348.

Gomez-Gil, B., Roque, A., Rotllant, G., Peinado, L., Romalde, J. L., Doce, A., Cabanillas-Beltrán, H., Chimetto, L. A., and Thompson, F. L., 2011. *Photobacterium swingsii* sp. nov., isolated from marine organisms. *International Journal of Systematic and Evolutionary Microbiology*, **61**: 315-319.

Hidalgo, R. B., Cleenwerck, I., Balboa, S., De Wachter, M., Thompson, F. L., Swings, J., De Vos, P., and Romalde, J. L., 2008. Diversity of vibrios associated with reared clams in Galicia (NW Spain). *Systematic and Applied Microbiology*, **31**: 215-222.

Huber, J. A., Welch, D. M., Morrison, H. G., Huse, S. M., Neal, P. R., Butterfield, D. A., and Sogin, M. L., 2007. Microbial population structures in the deep marine biosphere. *Science*, **318**: 97-100.

Kesarcodi-Watson, A., Miner, P., Nicolas, J. L., and Robert, R., 2012. Protective effect of four potential probiotics against pathogen-challenge of the larvae of three bivalves: Pacific oyster (*Crassostrea gigas*), flat oyster (*Ostrea edulis*) and scallop (*Pecten maximus*). *Aquaculture*, **344**: 29-34.

Kueh, C. S. W., and Chan, K. Y., 1985. Bacteria in bivalve shellfish with special reference to the oyster. *Journal of Applied Bacteriology*, **59**: 41-47.

Lambert, C., Nicolas, J. L., Cilia, V., and Corre, S., 1998. *Vibrio pectenicida* sp. nov., a pathogen of scallop (*Pecten maximus*) larvae. *International Journal of Systematic Bacteriology*, **48**: 481-487.

Lane, D. J., 1991. 16S/23S rRNA sequencing. In: *Nucleic Acid Techniques in Bacterial Systematic*. Stackebrandt, E., and Goodfellow, M. D., eds., John Wiley and Sons Press, Chichester, 115-175.

Lasa, A., Mira, A., Camelo-Castillo, A., Belda-Ferre, P., and Romalde, J. L., 2014. Analysis of the scallop microbiota by means of 16S rRNA gene pyrosequencing. *Frontier Marine Science Conference Abstract: IMMR/International Meeting on Marine Research 2014*, DOI: 10.3389/conf.FMARS.2014.02.00112.

Liang, B., Luo, M., Scott-Herridge, J., Semeniuk, C., Mendoza, M., Capina, R., Sheardown, B., Ji, H., Kimani, J., Ball, B. T., Van Domselaar, G., Graham, M., Tyler, S., Jones, S. J., and Plummer, F. A., 2011. A comparison of parallel pyrosequencing and Sanger clone-based sequencing and its impact on the characterization of the genetic diversity of HIV-1. *PLoS One*, **6**: e26745.

Liu, J. C., Sun, X. Y., Li M., Zhang, C. Y., Cao, S. Q., and Ma, Y. X., 2015. *Vibrio* infections associated with Yesso scallop (*Patinopecten yessoensis*) larval culture. *Journal of Shellfish Research*, **34**: 213-216.

McBain, A. J., Bartolo, R. G., Catrenich, C. E., Charbonneau, D., Ledder, R. G., Rickard, A. H., Symmons, S. A., and Gilbert, P., 2003. Microbial characterization of biofilms in domestic drains and the establishment of stable biofilm microcosms. *Applied and Environmental Microbiology*, **69**: 177-185.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, **41**: 590-596.

Riquelme, C. E., Araya, R., Vergara, N., Rojas, A., Guaita, M., and Candia, M., 1997. Potential probiotic strains in the culture of the Chilean scallop *Argopecten purpuratus* (Lamarck, 1819). *Aquaculture*, **154**: 17-26.

Riquelme, C. E., Chavez, P., Morales, Y., and Hayashida, G., 1994. Evidence for parental bacterial transfer to larvae in *Argopecten purpuratus* (Lamarck, 1819). *Biological Research*, **27**: 129-134.

Riquelme, C. E., Hayashida, G., Araya, R., Uchida, A., Satomi, M., and Ishida, Y., 1996. Isolation of a native bacterial strain from the scallop *Argopecten purpuratus* with inhibitory effects against pathogenic vibrios. *Journal of Shellfish Research*, **15**: 369-374.

Riquelme, C. E., Jorquera, M. A., Rosas, A. I., Avendaño, R. E., and Reyes, N., 2001. Addition of inhibitor-producing bacteria to mass cultures of *Argopecten purpuratus* larvae (Lamarck, 1819). *Aquaculture*, **192**: 111-119.

Riquelme, C., Hayashida, G., Toranzo, A. E., Vilches, J., and Chavez, P., 1995. Pathogenicity studies of a *Vibrio anguillarum* related (VAR) strain causing an epizootic in *Argopecten purpuratus* larvae cultured in Chile. *Diseases of Aquatic Organisms*, **22**: 135-141.

Romalde, J. L., Diéguez, A. L., Doce, A., Lasa, A., Balboa, S., López, C., and Beaz-Hidalgo, R., 2013. Advances in the knowledge of the microbiota associated with clams from natural beds. *Clam Fisheries and Aquaculture*, 163-190.

Sandaa, R. A., Magnesen, T., Torkildsen, L., and Bergh, Ø., 2003. Characterisation of the bacterial community associated with early stages of Great scallop, using denaturing gradient gel electrophoresis (DGGE). *Systematic and Applied Microbiology*, **26**: 302-311.

Schulze, A. D., Alabi, A. O., Tattersall-Sheldrake, A. R., and Miller, K. M., 2006. Bacterial diversity in a marine hatchery: Balance between pathogenic and potentially probiotic bacterial strains. *Aquaculture*, **256**: 50-73.

Shendure, J., and Ji, H., 2008. Next-generation DNA sequencing. *Nature Biotechnology*, **26**: 1135-1145.

Torkildsen, L., Lambert, C., Nylund, A., Magnesen, T., and Bergh, Ø., 2005. Bacteria associated with early life stages of the Great scallop, *Pecten maximus*: Impact on larval survival. *Aquaculture International*, **13**: 575-592.

Trabal, N., Mazón-Suástegui, J. M., Vázquez-Juárez, R., Asencio-Valle, F., Morales-Bojórquez, E., and Romero, J., 2012. Molecular analysis of bacterial microbiota associated with oysters (*Crassostrea gigas* and *Crassostrea corteziensis*) in different growth phases at two cultivation sites. *Microbial Ecology*, **64**: 555-569.

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, **73**: 5261-5267.

(Edited by Qiu Yantao)