Temporal Variation of Bacterial Community Structure in the Sea Surface Microlayer at Nearshore and Offshore Sites in the Northern Yellow Sea

Hongxia Ming[1], Shun Zhao[1,2], Jie Su[1], Yuan Jin[1] and Jingfeng Fan[1,3*]
[1]*Key Laboratory for Ecological Environment in Coastal Areas (SOA), National Marine Environmental Monitoring Center, Dalian China 116023.*
[2]*College of Fisheries and Life Science, Dalian Ocean University, Dalian China 116023.*
[3]*The Fourth Institute of Oceanography, SOA, Beihai China 536000.*
Email: jffan@nmemc.org.cn

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Abstract: Sea surface microlayers (SSM) are specific microbial habitats that are critical to global biochemical process. In this study, bacterial populations inhabiting the SSM from two types of coastal sites (nearshore vs. offshore) were investigated by culturing and DNA fingerprinting methods, and were compared with those of subsurface layer (SUB). Bacterial samples from the SSM were collected using a glass plate method during March, August, October and January in the Northern Yellow Sea. The total number of bacterial and culturable bacterial exhibited an obvious spatial distribution, with more abundant in the nearshore sites than the offshore sites, and they showed an obvious enrichment effect in the surface microlayer. DGGE and sequencing revealed a close relationship between the bacterial community in the surface microlayer and subsurface layer, with high similarities between the two dominant bacterial communities, Proteobacteria and Bacteroidetes. The bacterial abundance and diversity in the surface microlayer are obviously superior to those in the subsurface layer. It is more likely for bacterial communities to show significant differences with spatial and temporal changes.

1 INTRODUCTION

The ocean-atmosphere interface of 1~1000 μm is generally defined as sea surface microlayer (SML), covering more than 70% of the world's surface. Compared with the corresponding subsurface layer (SUB), the SML is a unique ecosystem with specific physical, chemical and biological properties (Cunliffe et al., 2013). Specially, SML plays an important role in sea-air gas exchange directly related to climate change, including carbon dioxide, carbon monoxide, dimethylsulfide and methane (Galgani et al., 2015).

Collectively, living organisms inhabiting within the SML are named as the neuston, and bacterial community within SML layer are referred to as the bacterioneuston (Franklin et al., 2005). Bacterioneuston has been considered to contain unique microbial species and populations (Maki, 2002). While regarding phylogenetic composition comparison of bacterioneuston and bacterioplankton, early studies that utilized molecular techniques offered conflicting conclusions. Some authors detected considerable different bacterial community structure between the bacterioneuston and bacterioplankton (Franklin et al., 2005), while others did not find the difference (Agogué et al., 2005).

From a taxonomic viewpoint, the bacterial community structure within the SML is poorly characterized, in total, covered limited region (Cunliffe et al., 2011). More studies that focus on a wider spatial range are need to gain a more comprehensive understanding of SML. To gain insight into bacterial community structure in the SML and to compare this with subsurface water, we utilized 16S rDNA gene DDGE sequencing to profile neustonic and planktonic bacterial diversity.

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2 MATERIAL AND METHODS

2.1 Sampling

2.1.1 Field Sites

Seawater samples of the surface microlayer and subsurface layer were collected from the North Yellow Sea in March (Spring), August (Summer), October (Autumn) and January (Winter). Samples were collected from four sites: A1 and B1 are nearshore sites where the main contamination sources are industrial effluents, aquaculture practices and urban activities; C1 and D1 are offshore sites which represent oligotrophic environment (Figure 1).

2.1.2 Sampling Methods

Samples of the SML water were collected using the glass plate method (Cunliffe and Wurl, 2015). Briefly, after disinfection treatment, a glass plate (300 ×400 ×5 mm) was vertically submerged in water, then it was lifted up at a speed of about 20 cm/s and the water films on both sides of the glass plate were scraped with a rubber wiper blade. About 50 mL SML water was quickly collected in a sterile bottle (Cunliffe et al. 2009). Samples of the subsurface layer water (200 mL) were collected from 0.5 m under the surface using a hydrophore.

2.2 Determination of the Total Number of Bacteria and Culturable Bacteria

The total number of bacteria was determined by flow cytometry. Briefly, 3 mL aliquots of seawater were fixed in 25% glutaraldehyde in a cryopreservation tube. The samples were then stained with Genefinder™ before determination. Following staining, the sample was allowed to stand for 10–15 min in the dark at room temperature, then, it was analyzed by flow cytometry (488 nm, 15 mW argon laser) with detection at 530/30 nm. Stained bacteria were discriminated and counted.

The total number of culturable bacteria in seawater was determined by the plate count method. Briefly, serial dilutions (1:10, 1:100) were prepared using sterilized seawater, after which 100 µl of each dilution was inoculated on three replicate plates of Marine Agar 2216. Colony forming units were counted after incubation at 25℃ for 1 to 2 weeks. Colonies were selected, picked and purified according to differences in colour and shape.

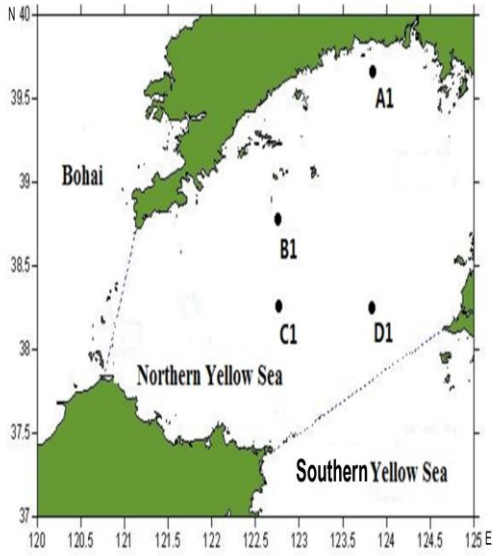

Figure 1: Schematic diagram of the survey stations in the Northern Yellow Sea.

2.3 Amplification of Bacterial V3 Region and Genotyping

DNA extraction from the membranes and bacteria strains were carried out according to the manufacturer's instructions of nucleic acid extraction (MOBIO). Products were purified using a PCR purification kit (Takara).

For DGGE, a nested PCR approach was employed by first conducting the reaction using primers 27F and 1492R, followed by PCR with primers 338F and 518R (Anja and Luisa, 2016). Primer 338F has a GC-clamp at the 5′-end. DGGE Gels were prepared using 8% (w/v) acrylamide/bisacrylamide with a 30% to 60% linear denaturant gradient. The gel was run in 1× TAE buffer at 60℃ for a total of 1000 V h (200 V, 5 h), then stained with Genefinder™. The PCR products with the appropriate forward PCR primer without the GC clamp were purified using a PCR purification kit (Takara, Japan), then ligated into pMD®19-T Vector (Takara, Japan) before being transformed into *Escherichia* coli DH5. Positive colonies were selected and sequenced.

2.4 Construction of Phylogenetic Tree

The sequences of the DGGE bands were investigated for homology and closest relatives in GenBank. Clustering analysis of the identified DNA sequences was conducted using Clustal X2.0 and MEGA 7, a phylogenetic tree was constructed by the Neighbor Joining method (NJ).

The microbial diversity of each sample was compared based on the number of electrophoretic bands using Quantity One (Bio Rad). The similarity dendrogram of DGGE banding patterns was calculated automatically and plotted in accordance with the UPGMA algorithm based on the Dice coefficient (Cs). The number of electrophoretic bands represents the richness index (S) of DNA sequences of the community.

3 RESULTS

3.1 Total Number of Bacteria and Culturable Bacteria

3.1.1 Total Number of Bacteria

The total number of bacteria was determined by flow cytometry (Figure 2). The concentration in the surface microlayer ranged from 4.84×10^5–8.90×10^6 CFU/mL with an average of 2.01×10^6 CFU/mL, which was significant higher than the subsurface layer ($P<0.05$). The total number of bacteria in the corresponding subsurface layer was 3.11×10^5–1.81×10^6 CFU/mL, with an average of 9.45×10^5 CFU/mL. The average bacterial concentration level in the surface microlayer was similar in spring, summer and autumn, with slightly lower in the winter. The maximum of the average total number of bacteria during the four seasons in the surface microlayer and subsurface layer both appeared at the nearshore station B1 (3.34×10^6 CFU/mL and 1.05×10^6 CFU/mL, respectively). However, the minima appeared at the offshore station D1 (1.08×10^6 CFU/mL and 7.07×10^5 CFU/mL, respectively).

3.1.2 Total Number of Culturable Bacteria

The total number of culturable bacteria in the surface microlayer ranged from 1.0×10^2–4.43×10^5 CFU/mL, with an average of 8.29×10^4 CFU/mL, while in the subsurface layer it ranged from 1.0×10^2–2.31×10^4 CFU/mL, with an average value of 6.28×10^3 CFU/mL. The total number of culturable bacteria in the surface microlayer at each station in each season was generally higher than that in the subsurface layer. For each season, the total numbers of culturable bacteria in the surface microlayer were similar in spring, summer and autumn, the concentration was about 3.5×10^4 CFU/mL. The level was slightly lower in the winter, which was 6.97×10^3 CFU/mL. The total number of culturable bacteria in the surface microlayer was 1.44×10^4 CFU/mL in autumn, which was slightly higher than other seasons (Spring and Summer was about 1.5×10^3 CFU/mL, when winter was 2.58×10^2 CFU/mL). The total numbers of culturable bacteria in the nearshore stations (A1 and B1) was much higher than that in the offshore stations (C1 and D1) in spring, summer and autumn. (Figure 3).

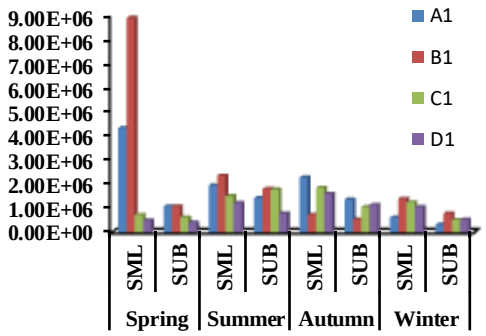

Figure 2: Diagram of variations in the total number of bacteria in the surface microlayer (SML) and subsurface layer (SUB).

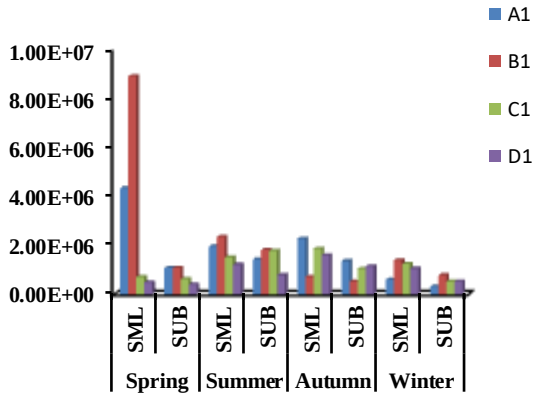

Figure 3: Diagram of the variations of the total number of culturable bacteria in the surface microlayer (SML) and subsurface layer (SUB).

3.2 PCR-DGGE Analysis

The amplified V3 region of bacterial 16S rDNA in the seawater samples from the surface microlayer and subsurface layer were analyzed. Distinguishable bands were purified and sequenced and the bacterial strains with the highest homology (greater than 99%) were identified.

3.2.1 Winter Samples

Total of 26 sequences were obtained in the winter (Figure 4a). There was a high similarity between the sequences of bacteria in the surface microlayer and that of the bacteria in six phylogenetic communities, Proteobacteria, Bacteroidetes, Bacillariophyta, Cyanobacteria, Actinobacteria and Firmicutes. There was a high similarity between the sequences of bacteria in the subsurface layer and that of bacteria in four phylogenetic communities, namely, Proteobacteria, Bacteroidetes, Bacillariophyta and Acidobacteria. Ten of the 26 sequences belonging to Proteobacteria in the surface microlayer (γ-Proteobacteria:4; α-Proteobacteria:4; β-Proteobacteria:2), three sequences belonging to Bacteroidetes (Flavobacterium spp.:1; Leeuwenhoekiella spp.:1; Zunongwangia spp.:1), one sequence belonging to Bacillariophyta, Cyanobacteria, Actinobacteria and Firmicutes, each. Besides, five sequences belonging to Proteobacteria in the subsurface layer (γ-Proteobacteria:5), two sequences belonging to Bacteroidetes (Flavobacterium spp.:1; Pseudoalteromonas spp.:1) one sequence belonging to Bacillariophyta, and one sequence of Acidobacteria (Figure 4a).

3.2.2 Spring Samples

Total of 27 sequences obtained in the spring (Figure 4b). There was a high similarity between the sequences of bacteria in the surface microlayer and those in Proteobacteria, Bacteroidetes, Cyanobacteria and Verrucomicrobia. There was also a high similarity between the sequences of bacteria in the subsurface layer and those of bacteria in Proteobacteria and Bacteroidetes. Eleven of 27 sequences belonging to Proteobacteria in the surface microlayer (γ-Proteobacteria:9; α-Proteobacteria:2), three sequences belonging to Bacteroidetes (Flavobacterium spp.:1; Gramella spp.:1; Polaribacter spp.:1), one sequence belonging to Cyanophyta and one to Verrucomicrobia. There were six sequences belonging to Proteobacteria in the subsurface layer (γ-Proteobacteria:5; α-Proteobacteria:1), and five sequences belonging to Bacteroidetes (Flavobacterium spp.:2; Bacillus spp.:2; Chlorobium spp.:1) (Figure 5b).

3.2.3 Summer Samples

Total of 33 sequences obtained in the summer (Figure 4c). There was a high similarity between the sequences of bacteria in the surface microlayer and those of Proteobacteria, Bacteroidetes, Bacillariophyta, Cyanobacteria and Firmicutes. There was also a high similarity between sequences of bacteria in the subsurface layer and those of Proteobacteria, Bacteroidetes, Bacillariophyta, Firmicutes and acidobacteria. There were 11 sequences belonging to Proteobacteria in the surface microlayer (γ-Proteobacteria:8; α-Proteobacteria:3). There were five sequences belonging to Bacteroidetes (Flavobacterium spp.:2; Gramella spp.:1; Polaribacter spp.:1; Leeuwenhoekiella spp.:1). There was one sequence belonging to Bacillariophyta, two similar to Cyanophyta, and one to Firmicutes. There were seven sequences belonging to Proteobacteria in the subsurface layer (γ-Proteobacteria). There were three sequences belonging to Bacteroidetes (Flavobacterium spp.:2; Polaribacter spp.:1). There was one sequence belonging to Bacillariophyta, one to Firmicutes and one to Acidobacteria (Figure 5c).

3.2.4 Autumn Samples

Total of 26 sequences obtained in the autumn (Figure 4d). There was a high similarity of the sequences of bacteria in the surface microlayer and subsurface layer to those of Proteobacteria and Bacteroidetes, Bacillariophyta and Firmicutes. Ten of 26 sequences belonging to Proteobacteria in the surface microlayer (γ-Proteobacteria:8; α-Proteobacteria:2), five sequences belonging to Bacteroidetes (Flavobacterium spp.:3; Gramella spp.:2), one sequence belonging to Bacillariophyta and one belonging to Firmicutes. Besides, there were five sequences belonging to Proteobacteria in the subsurface layer (r- Proteus:3; α-Proteobacteria:2), two sequences belonging to Bacteroidetes (Polaribacter spp. and fusobacterium spp.), one sequence belonging to Bacillariophyta, and one to Firmicutes (Figure 5d).

3.2.5 Clustering Analysis

Clustering analysis (Figure 4) revealed that the bacterial communities in the surface microlayer and subsurface layer at the corresponding stations were clustered into one branch in all seasons except winter. However, the bacteria in the surface microlayer at the stations were clustered into one branch in winter, while those in the subsurface layer were clustered into one branch.

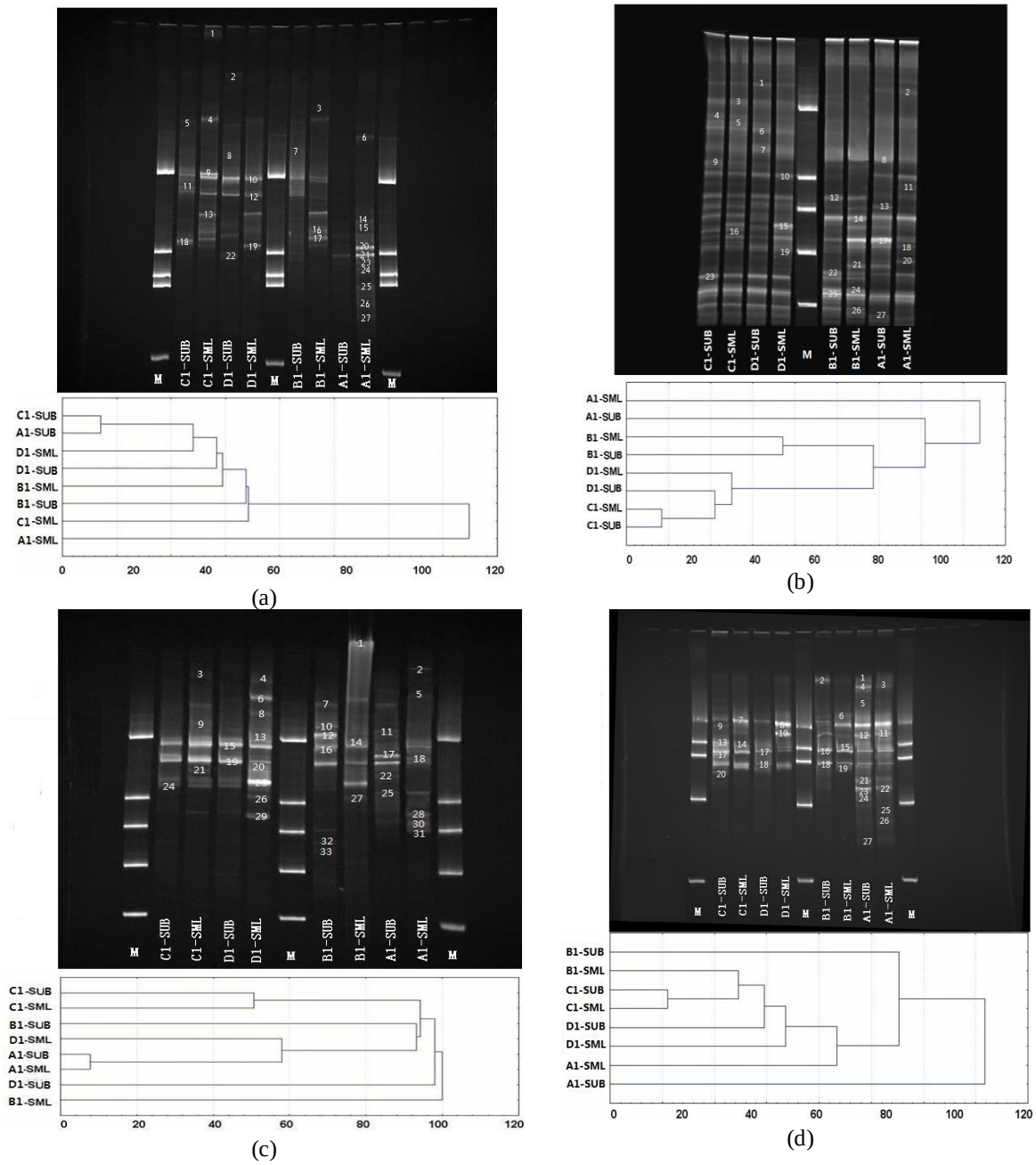

Figure 4: DGGE Profiles of V3 Region of Bacterial 16S rDNA (bands marked by numbers were sequenced) Figure a, b, c, d represent Winter, Spring, Summer and Autumn samples, respectively.

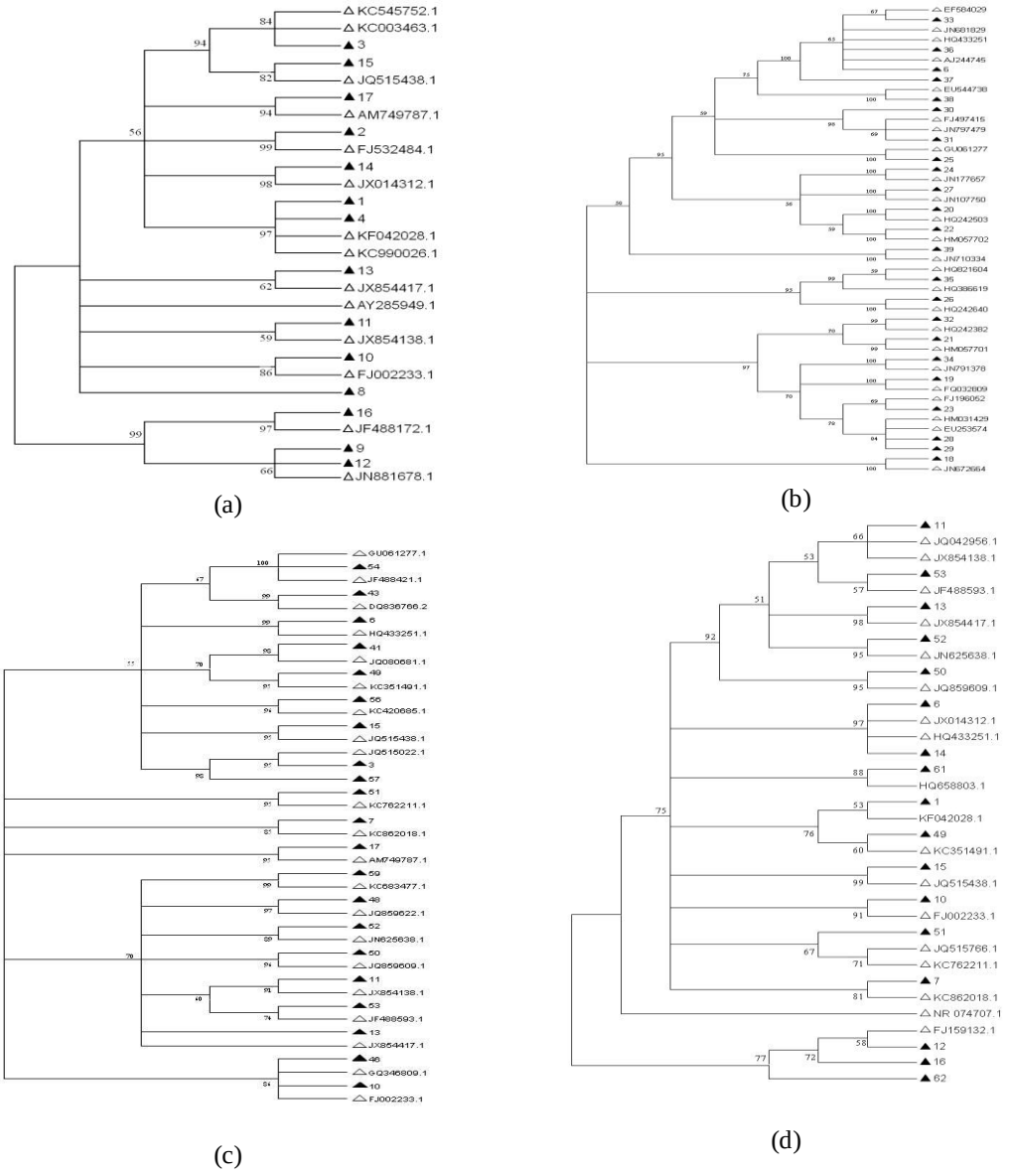

Figure 5: PCR-DGGE Phylogenetic Tree Based on the V3 Region of Bacterial 16S rDNA Figure a, b, c, d represent Winter, Spring, Summer and Autumn samples, respectively.

Note: Bands recovered by cutting the gel; △: known sequences in database

4 DISCUSSIONS

We analyzed whether such differences would vary with spatial and temporal changes. There were high bacterial abundances in the seawater of the surface microlayer and subsurface layer in summer and autumn. The total number of bacteria in the surface microlayer was the same level in spring, while that in the subsurface layer was the same low level in spring and winter. This may be because the relatively mild environment is more suitable for the growth of most bacteria, resulting in the low total number of bacteria in the colder environment during winter. When the temperature increases in spring, bacteria begin to propagate rapidly. The bacteria in the surface microlayer have more sensitive changes

than those in the subsurface because they receive more solar radiation and wind transport, as well as due to the properties of the surface microlayer itself. As a result, the number of bacteria in the surface microlayer quickly reaches a high level during spring, then remains stable until the weather becomes colder in winter. Similarly, the total number of culturable bacteria in the surface microlayer and subsurface layer in the four seasons conform to this trend.

Thus, when compared with bacteria in the subsurface layer, those in the surface microlayer are more sensitive to changes with time. There may also be a significant difference in the properties of the surface microlayer with changes in space (Hervas and Emilio, 2009). The results of the present study indicated that the average values of the total number of bacteria in the surface microlayer at the same station were much higher than those in the subsurface layer. However, there was an obvious enrichment effect in the surface microlayer of seawater in the offshore region with relatively poor nutrition. Evaluation of the total number of culturable bacteria revealed a more obvious enrichment effect in the surface microlayer of seawater in the nearshore region. Therefore, the bacterial abundance of the surface microlayer and subsurface layer presents different variation trends with changes of time and space. This may be because the nearshore region is more affected by the surface runoff and human factors, resulting in enrichment of a large amount of organic and inorganic substances in the surface microlayer, providing a good environment for the survival of microorganisms. But, the enrichment of organic and inorganic substances in the surface microlayer also would be a deterrent to bacterial growth. Perhaps this is why the surface microlayer bacteria abundance in B1 station was much higher than that in A1 station.

Based on the above analysis, there is a difference in the bacterial abundance of the surface microlayer and subsurface layer. Therefore, we further explored the response of the bacterial communities in the surface microlayer and subsurface layer to different times and space by DGGE, cloning and sequencing. The results revealed the same dominant bacteria in the surface microlayer and subsurface layer. For example, there were a large number of sequences of bacteria in the surface microlayer and subsurface with extremely high similarities to those of Proteobacteria and Bacteroidetes. Furthermore, there were a few sequences of bacteria that showed extremely high similarities to those of Bacillariophyta and Firmicutes. This similarity may have been due to biological sources in the surface microlayer developing by the upward transport of organisms caused by the physical process, rather development in situ (Anja and Luisa, 2016). The bacteria in the subsurface layer can enter into the surface microlayer through the effects of TEP rising and seawater surface tension. The bacteria in the surface microlayer also enter into the subsurface layer through the effects of aerosol and wind force.

However, spatial and temporal factors resulted in an obvious difference in the composition of bacterial communities in the surface microlayer and subsurface layer. On the spatial scale, bacterial groups in the surface microlayer differed from those in the subsurface layer in the nearshore regions. Sequences in the subsurface layer were different from those in the surface microlayer. The sequences of bacteria in the surface microlayer in the offshore region had high similarities to those of Cyanobacteria, Verrucomicrobium, α-Proteobacteria and Firmicutes, while Aerococcus was detected in the surface microlayer and Eubacterium in the subsurface layer, which is consistent with the results of previous studies. Overall, these findings indicate that the structure of bacterial communities in the surface microlayer and subsurface layer vary with water environments (Wurl et al., 2009). Analysis of these differences revealed that special functional communities may be formed in this unique habitat of the surface microlayer. Specifically, unique bacterial communities that are stable and different from those in the corresponding subsurface layer can be formed in the surface microlayer under the action of TEP, wind force and enrichment effects. Bacterial communities in the surface microlayer and subsurface layer change due to the influence of spatial and temporal factors, resulting in a great difference between the bacterial communities.

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