

doi: 10.1093/femsle/fnv106 Advance Access Publication Date: 16 July 2015 Research Letter

RESEARCH LETTER - Environmental Microbiology

Diversity of bacterioplankton in the surface seawaters of Drake Passage near the Chinese Antarctic station

Mengxin Xing, Zhao Li, Wei Wang and Mi Sun*

Key Laboratory of Sustainable Development of Marine Fisheries, Ministry of Agriculture, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, PR China

*Corresponding author: Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 106 Nanjing Road, Qingdao 266071, Shandong Province, PR China. Tel: +86-532-85819525; Fax: +86-532-85841193; E-mail: sunmi0532@yahoo.com One sentence summary: Marine bacterioplankton diversity in the surface waters of Drake Passage. Editor: Yu-Zhong Zhang

ABSTRACT

The determination of relative abundances and distribution of different bacterial groups is a critical step toward understanding the functions of various bacteria and its surrounding environment. Few studies focus on the taxonomic composition and functional diversity of microbial communities in Drake Passage. In this study, marine bacterioplankton communities from surface seawaters at five locations in Drake Passage were examined by 16S rRNA gene sequence analyses. The results indicated that psychrophilic bacteria were the most abundant group in Drake Passage, and mainly made up of Bacillus, Aeromonas, Psychrobacter, Pseudomonas and Halomonas. Diversity analysis showed that surface seawater communities had no significant correlation with latitudinal gradient. Additionally, a clear difference among five surface seawater communities was evident, with 1.8% OTUs (only two) belonged to Bacillus consistent across five locations and 71% OTUs (80) existed in only one location. However, the few cosmopolitans had the largest population sizes. Our results support the hypothesis that the dominant bacterial groups appear to be analogous between geographical sites, but significant differences may be detected among rare bacterial groups. The microbial diversity of surface seawaters would be liable to be affected by environmental factors.

Keywords: Antarctic; Drake Passage; bacterial diversity; surface seawater; 16S rRNA

INTRODUCTION

Antarctic environments are extraordinary in the harshness of their climates, such as drought, high ultraviolet (UV) radiation, light limitation, violent storms and extremely low temperatures (Tytgat et al. 2014). For withstanding the selective pressure, Antarctic insects and mammalian herbivores are generally absent and microorganisms play a critical role in the biogeochemical cycling of Antarctic ecosystem (Murray et al. 1998; Yerqeau et al. 2009). According to different temperature limitation of cell growth, the psychrophilic microorganisms in Antarctic environments are mainly divided into psychrophiles and psychrotrophs, and both of them can survive at 0°C. The psychrophiles prefer

to thrive at temperature below 15°C, while the optimum temperature of psychrotrophs is 20–30°C (Morita 1975). The growth of microorganisms exposed to extremely low temperature is challenged by several factors including reduced enzymatic reaction rates, limited bioavailability of nutrients, and extreme pH or salinity. To thrive successfully in cold environments, the psychrophilic microorganisms have evolved a complex range of structural and functional adaptations (Margesin and Miteva

For two centuries, many animal and plant species do not exhibit geographic patterns like the latitudinal gradient of increasing species richness from polar to equatorial regions (Hillebrand 2004; Pommier et al. 2007). Due to small size, great abundance

and easy dispersal, it is suggested that bacteria would also show little or no latitudinal gradient of diversity (Fuhrman et al. 2008). The geographical patterns lack a consensus explanation. Some studies show that geographical differences can be found in the structure and composition of marine bacterioplankton populations (Jamieson et al. 2012), while other studies demonstrate that the geographical parameters are not correlated with bacterioplankton distribution (Zubkov et al. 2002). In Antarctic surface seawaters, especially in Drake Passage, however, few studies focus on the relative investigations.

Drake Passage is located between the southern extension of the South American shelf and the Western Antarctic Peninsula shelf. Because of extreme environments, such as large waves, fierce storms and strong currents, sampling in Drake Passage is very difficult and therefore infrequent (Waller et al. 2011). Compared to other Antarctic environments, only limited information about the diversity and function of microbial communities in Drake Passage is available. In summer (2012) cruises in Drake Passage near Great Wall Station, we have investigated the diversity of marine bacteioplanktons, the dominant psychrophilic microorganisms and geographic pattern of five surface seawater communities with different locations. Our aims are to isolate the main psychrophilic microorganisms, describe and compare the bacteioplankton compositions with different geographical sites, demonstrate the correlation between microbial diversity and locations, and enhance understanding of diversity and activity of bacterioplankton communities in surface seawaters of Drake Passage near the Great Wall Station, Antarctic.

MATERIALS AND METHODS

Sample collection

Field measurements and sample collections were conducted in March 2012 during the 28th Chinese National Antarctic Research Expedition. Five surface seawater samples (5 m depth) with different locations (A1-A5) were collected from Drake Passage (Fig. 1) near the Great Wall Station (62°12′59′S, 58°57′52′W) in Antarctic. Bacteria existed in the surface seawater samples were collected by filtration of 4 L of seawater onto 0.2 μ m pore size filter (Pall, Lane Cove, Australia). All the samples were stored at -80°C until further processing.

DNA extraction, 16S rRNA clone library construction, and restriction fragment length polymorphism

Genomic DNA was extracted in duplicate to avoid bias from five samples (A1-A5) using a QiAamp DNA stool Mini Kit (Qiagen, Germany). The PCRs were carried out in septuplicate 25 μ L reactions with 50–80 ng of genomic DNA, 2.5 μ L 10 imes PCR buffer, 2 μ L 10 mM dNTP, 0.2 μ L Taq polymerase (Takara, Japan), 19.8 μ L ddH₂O, 0.5 μ L 10 μ M Bact 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and Bact 1492R (5'-GGTTACCTTGTTACGACTT-3'). The amplification program consisted of an initial denaturation step at 95°C for 5 min, followed by 34 cycles of 95°C for 30 s, 55°C for 45 s and 72°C for 90 s, and a final extension period of 10 min at 72°C (Xing et al. 2013). Replicate PCR products were pooled, purified, cloned into PMD18-T vector (Takara, Japan) and transformed into Trans 5α (Tiangen, China), respectively. About 200–300 positive colonies from each sample were chosen by chance. Products of the 27F-1492R 16S rRNA gene PCR amplification were digested with the restriction endonucleases BsuRI and Hin6I (Fermentas, Canada). The same restriction fragment length polymorphism (RFLP) patterns were clustered into operational taxonomic units (OTUs). Three clones of each OTU were chosen at random and plasmid inserts were sequenced bidirectionally with M13 primers (Xing et al. 2013). All experiments described above were repeated three

Statistical and bioinformatics analysis

Bidirectional sequences were assembled with DNAStar and trimmed to remove vector and low-quality sequences using the PhredPhrap script. Chimeras were removed using Bellerophon software (Huber, Faulkner and Hugenholtz 2004), implemented at the Greengenes website (http://greengenes.lbl.gov) (DeSantis et al. 2006). The 16S rRNA gene sequences which passed the quality and chimera filters were used in the subsequent analyses. Each representative sequence of OTUs was submitted to



Figure 1. Location of sampling stations in Drake Passage near the Great Wall Station, Antarctic. Sampling coordinates: A1(64°00'S, 65°59'W), A2 (63°01'S, 65°02'W), A3 (62°59'S, 64°02'W), A4 (62°34'S, 61°58'W) and A5(62°00'S, 61°01'W).

Table 1. Clone library coverage and bacterial diversity indices for the 16S rRNA gene clone libraries constructed from five samples (A1-A5).

Samples	No. of OTUs	No. of clones	Coverage%	Chao1	ACE	Jackknife	Shannon	Simpson
A1	38	269	89.47	35.46	36.82	37.58	2.54	0.17
A2	32	256	87.50	27.54	29.33	31.00	2.21	0.19
A3	33	234	90.90	31.30	32.49	34.00	2.84	0.09
A4	14	184	92.86	11.00	11.58	12.00	1.29	0.44
A5	48	256	89.58	42.33	43.99	38.87	3.03	0.09

GenBank and granted an accession number. Taxonomy was assigned to each OTU using the Ribosomal Database Project (RDP) classifier with a minimum threshold of 80% (Pope et al. 2012) and BLAST method. The representative sequence for each OTU with <97% identity to any sequence in GenBank database was defined as previously undescribed OTUs (UOTUs) (Xing et al. 2013). Mothur was used to describe species diversity with a threshold of 97%. The richness indices included Chao1, the abundancebased coverage (ACE) and interpolated jackknife, and the diversity indices contained the Shannon-Weaver diversity index and Simpson reciprocal diversity (Schloss et al. 2009). Good's coverage and rarefaction analysis for the five libraries were also determined. Cluster analysis of the community composition was performed using PAST with a correlation matrix (Zeng et al. 2014).

RESULTS

Sequence generation

A total of 1199 positive clones were picked from the five 16S rRNA clone libraries (A1-A5) and 112 OTUs were obtained through RFLP analysis. These near-full-length sequences (OTUs) were assigned to six different phyla or groups (including Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Verrucomicrobia and unclassified groups), and were deposited into the GenBank database with accession numbers KP975257-KP975368. Each of the 16S rRNA clone libraries contained 184-256 positive clones, with OTUs ranging from 14 to 48. Good's coverage estimations revealed that 87.5-92.86% of species were obtained in all of the five samples and the rarefaction curves tended to approach the saturation plateau (see Fig. S1, Supporting Information). The diversity indices showed that A5 and A4 communities appeared to have higher and lower richness as compared to other communities, respectively (Table 1).

Bacterioplankton species diversity

The majority of OTUs (68%) showed ≥97% sequence similarity to known taxa in GenBank database. At the phylum level, cluster analysis of bacterial composition revealed a conservation of the community composition (>78% similarity) in all of the libraries except the A4 library which was <50% similar with other libraries (Fig. 2a). RFLP analysis revealed that the A1 library contained the maximum number of phyla (6), where Firmicutes, Proteobacteria and Actinobacteira were the most dominant groups and accounted for 97.4% of the OTU sequences (Fig. 3a). Both the A2 and A3 libraries showed relatively simple diversity wherein Proteobacteria and Firmicutes represented 91% and 98.7% of the OTU sequences, respectively. Although the number of phyla of A4 library was similar with A2 and A3 library, Firmicutes was the only dominant phylum in A4 library which accounted for 86.4% of the OTU sequences (Fig. 3a). Furthermore, Proteobacterial diversity analysis at the family level

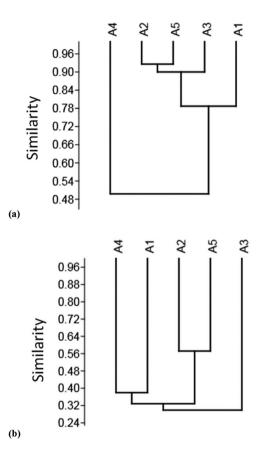


Figure 2. Sample sorting analysis. (a) Cluster analysis dendrogram showing percentage similarity of bacterial diversity at the phylum level in the five sampling sites based on Bray-Curtis resemblance of the relative abundance of bacterial groups. (b) Cluster analysis dendrogram showing percentage similarity of OTUs in the five sampling sites based on Bray-Curtis index.

indicated that Gammaproteobacteria was the principal contributor to the observed dissimilarity between A4 library and other libraries (Fig. 3b).

Dominant bacteioplanktons and psychrophilic bacteria

The 10 most abundant OTUs within the five samples were determined to understand further the important bacteria. Dominant OTUs belonged to Bacillus (23.05-79.90%) were detected in all five libraries, and abundant OTU sequences related to Aeromonas (4.09-28.91%) and Psychrobacter (1.56-4.30%) were found in A1, A2, A4 and A5 library. In addition to these species, the most abundant OTUs associated with the A1 library were sequences related to Pelomonas (12.27%), Curtobacterium (7.81%), Beijerinckia (1.86%) and Brevibacillus (1.86%). The A2 library consisted mainly of sequences related to Candidatus (6.25%), Rhodococcus

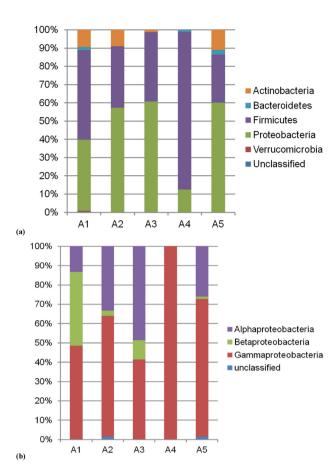


Figure 3. Baterial composition of the different communities. (a) Relative distribution of the bacterial phyla in the different locations. (b) Relative distribution of Proteobacteria in the different locations. Sequences that could not be classified into any known group were assigned as 'unclassified'.

(1.95%-5.86%), Planktomarina (3.91%), Phyllobacterium (1.56%) and Pantoea (1.56%); the A3 library consisted mainly of Candidatus (10.68%), Swaminathania (6.84%), Stenotrophomonas (6.41%), Planktomarina (4.27%), Achromobacter (2.99%), and two UOTUs belonged to Gammaproteobacteria (6.41%) and Alphaproteobacteria (3.85%), respectively; the most abundant sequences in the A4 library were those related to Pseudomonas (4.35%), Fictibacillus (2.17%) and Elizabethkingia (1.09%); for the A5 library, it was numerically contained sequences related to Candidatus (4.69%), Rhodococcus (4.69%), Cobetia (3.52%), Planktomarina (3.13%) and Halomonas (2.34–2.73%) (see Table S1, Supporting Information).

In the present work, Gammaproteobacteria which was recognized as the main psychrophilic bacteria in ocean environments dominated in all five libraries (Fig. 3b). Among them, Aeromonas and Psychrobacter were detected in five libraries (2.56-30.86% and 1.56-7.42%, respectively); Pseudomonas existed in the A1, A3 and A4 library (1.12, 0.85 and 4.35%, respectively); Halomonas was identified in A1, A3 and A5 library (0.74, 1.28 and 7.03%, respectively); Acinetobacter, Cobetia and Marinomonas were found in the A1 and A5 library (1.12 and 0.78%, 0.74 and 5.08%, 1.12 and 1.95%, respectively) (Table 2). Furthermore, Bacillus in Firmicutes which was also psychrophilic bacteria had been detected abundant in five surface seawater libraries (described above).

Thirty-six OTU sequences showed only 86-96% sequence similarity to known taxa in GenBank database (see Table S2, Supporting Information) and were defined as UOTUs. The major-

Table 2. The main psychrophilic bacteria present in five libraries (A1-A5)*

Group	A1	A2	А3	A4	A5
Psychrobacter	11/1	4/1	4/2	3/1	19/5
Aeromonas	15/2	79/3	6/1	12/4	39/3
Pseudomonas	3/1	0/0	2/1	8/1	0/0
Halomonas	2/1	0/0	3/2	0/0	18/4
Acinetobacter	3/1	0/0	0/0	0/0	2/1
Cobetia	2/1	0/0	0/0	0/0	13/3
Marinomonas	3/1	0/0	0/0	0/0	5/2

^{*}Numbers below the diagonal line represent the number of OTUs in five libraries. whereas numbers above the diagonal line indicated the number of clones in five libraries

ity of UOTUs originated from A2 and A5 library. A5 library contained the maximum number of UOTUs (13) and the sequences mainly belonged to Gammaproteobacteria (6). Alphaprotebacteria (2), Bacilli (2) and Actinobacteria (2). A2 library contained 12 UO-TUs which were mainly related to Alphaproteobacteria (7) and Gammaproteobacteria (2). Only one UOTU was identified in A4 library on the contrary. At the genus level, the sequences of five UOTUs (13.89%) had relatively high similarity with psychrophilic bacteria including Psychrobacter (4) and Cobetia (1), while the sequences of three UOTUs were similar with Candidatus (SAR11) (see Table S2, Supporting Information).

Unique and shared OTUs among five bacterial communities of surface seawater in Drake Passage

Only 2 of the 112 identified OTUs (1.8%) were cosmopolitans, i.e. they were found at all libraries, belonging to Bacillus. In contrast, 80 OTUs (71%) were endemic, i.e. they were only found at one of surface seawater libraries, 18 OTUs (16%) were found in two of the libraries and only a small fraction (12) of all OTUs were found in three or four libraries (see Fig. S2a, Supporting Information). Comparing percentage similarity of OTUs composition in different samples using dendrogram indicated that five libraries were dissimilar to each other (less than 40%) except for A2 and A5 library with relatively high similarity (more than 56%) (Fig. 2b). However, the frequencies of the 80 endemic OTUs were mostly (65%) very low, with one or two representative sequences, and a few OTUs (28) contained from 3 to 15 sequences per library (see Fig. S2b, Supporting Information). Furthermore, the sequences of shared OTUs between five libraries mainly belonged to Gammaproteobacteria, Alphaproteobacteria and Firmicutes, and the percentage of sequences originated from shared OTUs in each library was very high, ranging from 66.24 to 91.58% (Table 3).

DISCUSSION

Taxonomy and comparison of bacterioplankton communities

Among the members of Proteobacteria, Gammaproteobacteria, Alphaproteobacteria and Betaproteobacteria dominated in all five communities of surface seawaters except the A4 community wherein only Gammaproteobacteria was abundant (Fig. 3b). This pattern of dominance has already been reported in the west of the Antarctic Peninsula (Murray and Grzymski 2007) although the surface water of Scotia Sea, Alphaproteobacteria has been found to be the dominant group followed by

Table 3. Shared phyla/classes between five bacterial communities of surface seawater in Antarctic.

	Share OTUs					
Phylum/class		A1	A2	A3	A4	A5
Bacteroidetes	1	3	0	0	2	0
Actinobacteria	4	23	20	3	0	23
Alphaproteobacteria	8	2	41	55	0	34
Betaproteobacteria	1	33	2	0	0	0
Gammaproteobacteria	11	40	83	28	9	78
Firmicutes	7	111	82	69	158	63
Total	32	212	228	155	169	198
Clone#/total clones (%)		78.81	89.06	66.24	91.85	77.34

^{*}Number of clones belonging to the shared OTUs.

Gammaproteobacteria (Zinger et al. 2011). In a global distribution study, Gammaproteobacteria generally dominates in both pelagic and benthic ecosystems (Jamieson et al. 2012). Among the Alphaproteobacteria, SAR11 clade (Candidatus species) was abundant in A2, A3 and A5 community (see Table S1, Supporting Information), which was in accordance with a previous research which suggested that 2.4×10^{28} SAR11 cell existed globally in the oceans and half of them were located in the euphotic zone (Morris et al. 2002). However, Archaea was not detected in any communities in this study. According to previous research on Drake Passage, archaeal community richness was commonly lower in summer than in winter (Manganelli et al. 2009). The PCR primer in the present study which was mainly used to detect 16S rRNA genes of bacterial groups would be also difficult to discover the genes of Archaea. The specific primer of Archaea will be employed to identify the structure and diversity of Archaea in the future.

Bacteroidetes was only detected in A1, A4 and A5 community with low proportion (1.1-2.7%). This finding was in accordance with previous studies regarding the marine bacterioplankton diversity of the Scotia Arc with low chlorophyll α concentration (Jamieson et al. 2012). However, Zeng et al. (2014) demonstrated that Bacteroidetes was dominant in surface seawaters of Ardley Cove and Great Wall Cove, consistent with high concentrations of chlorophyll α and particulate organic carbon. Bacteroidetes members have the capability to degrade polymeric substances in the ocean, while Gammaproteobacteria and Alphaproteobacteria seem better adapted to use monomers rather than polymers (Cottrell and kirchman 2000b; González et al. 2008). In this study, lower Bacteroidetes and higher Gammaproteobacteria and Alphaproteobacteria were found in five bacterial communities, suggesting the low concentration of polymeric substances in the surface seawater of Drake Passage. These may be the reasons that can partly explain the high similarity between A2 and A5 community (Fig. 2). Similar proportions of Gammaproteobacteria and Alphaproteobacteria in A2 and A5 community (Fig. 3b) may attribute to similar marine environments. The results above were also in agreement with a previous research, which suggested that bacterioplankton community structure could be used as an indicator of marine ecosystem status (Zeng et al. 2014).

Cluster analysis showed that A4 community had low similarity with other communities (Fig. 2a) and Firmicutes was the only abundant phylum in A4 community (Fig. 3a). Previously, Firmicutes was detected in coastal sediments with high proportion and Bacilli in Firmicutes had been identified as indicators of human fecal contaminations in watersheds (Zinger et al. 2011). Some investigations also suggested that coastal environments

including coastal waters and sediments were subjected to increasing nutrient, freshwater, organic matter and pollution originated from land (Rappé, Vergin and Giovannoni 2000; Halpern et al. 2008). According to the locations of sampling in this study, A4 was closest to the coast (Fig. 1). The taxonomic differences between A4 community and other communities might result from the influence of land.

Psychrophilic bacteria

The last decade has been a growing interest in psychrophilic bacteria (Margesin and Miteva 2011). Various groups of bacteria belonging to α -, β -, γ -proteobacteria and Cytophaga-Flavobacterium-Bacteriodes phylum have been reported to survive in permanently cold environments, such as Arctic soils and sediments, Antarctic lakes and oceans (Gilbert et al. 2004; Männistö and Häggblom 2006; Zeng et al. 2014). For surviving in extremely cold environments, psychrophilic bacteria have often evolved specific biosynthetic pathways to overcome all the adversities (D'Amico et al. 2006), including maintenance of functional membranes, cold-adapted enzymes and cold-shock or heatshock responses (Maiangwa et al. 2015). In polar ocean environments, psychrophilic bacteria are also halophilic and halotolerant (Oren 2015). In this study, psychrophilic bacteria were the main bacterioplankton groups in surface seawater communities of Drake Passage. Of the 10 most abundant bacterial OTUs, several were related to known psychrophilic bacteria wherein Bacillus, Psychrobacter and Aeromonas were dominant (see Table 3 and Table S1, Supporting Information). Psychrobacter, which is commonly observed in permanently cold environments (Jamieson et al. 2012), is also halotolerant (Juni and Heym 1986) and some species (such as Psychrobacter sp. strain G) can produce extracellular lipolytic enzymes with cold activity (Che et al. 2013). Previous investigations on Bacillus and Aeromonas in Antarctic disclosed that they were capable of producing cold-active glycosyl hydrolases, such as β -glucosidases, proteases and celulases (Dong et al. 2013; Cristóbal et al. 2015).

Moreover, other psychrophilic bacteria which were detected in surface seawater communities also had the ability to surviving in cold ecosystems. Pseudomonas species are the most commonly reported psychrophilic bacteria (D'Amico et al. 2006). In addition to cold-active lipase, antifreeze proteins are also produced by Pseudomonas to deal with low temperature (Muryoi et al. 2004; Mohamad et al. 2013). Cold-active lipases have been reported from comparatively fewer species of Halomonas which are mainly halotolerant bacteria in surface seawaters (Kaye et al. 2004; Jadhav et al. 2013). Cobetia can produce a high amount of trans-unsaturated fatty acids at low growth temperature

(Yumoto et al. 2004), while Acinetobacter produce cold-active esterase and lipase (Zheng et al. 2011; Kim et al. 2012). Additionally, UOTUs analysis indicated that the sequences which had relatively high similarity with psychrophilic bacteria accounted for high percentage of all UOTUs (13.89%). This finding is in accordance with the survey on novel bacterial and fungal species and genera from various cold habitats, which demonstrates that psychrophilic bacteria represent a vast resource of novel microorganisms (Margesin and Miteva 2011).

The determination of the numbers and relative abundances of different bacterial groups is a critical step toward understanding the functions of various bacteria (Cottrell and Kirchman 2000a; Zeng et al. 2014). In the present work, psychrophilic bacteria were most abundant in surface seawater communities, suggesting that psychrophilic bacteria played a critical role in the biocycling of Drake Passage near the Great Wall Station, Antarctic. Although psychrotrophs and psychrophiles were not determined with respect to individual bacterial species, the results of this study increased the knowledge of psychrophilic bacteria in Drake Passage, the function and cold-adapted mechanism of specific psychrotrophs or psychrophiles need to be investigated

In addition to psychrophilic bacteria, some abundant OTUs recovered were related to Rhodococcus and Stenotrophomonas. Many investigations have demonstrated the resistance of Rhodococcus and Stenotrophomonas species to UV radiation in Antarctic (Gouvêa Taketani et al. 2013; Vasileva-Tonkova et al. 2014). Species of Bacillus which were main psychrophilic bacteria in surface seawater communities (described above) were also proved to be UV-resistant bacteria (Wang et al. 2011). Although Antarctic ozone hole and the consequent increase of UV radiation could be particularly deleterious for bacteria, aerobic bacteria in Antarctic surface seawater have high resistance and repair capabilities against UV radiation damage (Wang et al. 2011; Vasileva-Tonkova et al. 2014). Characterization of microbial diversity of surface seawaters in Antarctic would be of considerable relevance for assessing climatic effects on microbial com-

Latitudinal gradient and degree of shared/unique bacteria

To date, whether microbial diversity has correlation with latitudinal gradient is still uncertain. In this study, diversity for surface seawater communities did not correlate with latitude, i.e. microbial diversity across locations showed no apparent geographical pattern (Table 1). This finding is in accordance with the research on surface and deep marine bacterial communities in Antarctic and Arctic regions, which suggest that environmental factors rather than geographical isolation are the main determinant of the bacterial community composition in surface waters (Ghiglione et al. 2012; Zeng et al. 2014). On the contrary, other investigations on marine bacterioplankton show a significant latitudinal gradient in diversity, and correlations with environmental factors are much weaker (Pommier et al. 2007; Fuhrman et al. 2008). Although Pommier et al. (2007) showed latitudinal gradient in species diversity, sampling was restricted to coastal seawaters and high-latitude samples were only detected from the Northern Hemisphere. Sampling in the present work was also restricted to Drake Passage closed to Great Wall Station, and the number of sampling was relatively low. To conclusively determine the correlation between latitude and microbial diversity, more data need to be obtained from additional locations. Moreover, Ghiglione et al. (2012) revealed that the microbial diversity of surface seawaters was vulnerable to environmental influences. Special environmental features of Drake Passage would be the main reason for microbial diversity of surface seawaters. Thus, the correlation between microbial diversity and environmental factors, such as primary production, temperature, chlorophyll concentration, water properties and anthropogenic factors (Ghiglione et al. 2012; Sul et al. 2013), need to be demonstrated further.

Although low degree of cosmopolitans and high degree of endemisms were observed in different communities of Drake Passage and little similarity at the OTU level was detected between the different communities, cosmopolitans had the largest population sizes. These findings were in accordance with previous study regarding the cosmopolitans/endemic microorganisms and different distribution of bacterial groups in the surface ocean (Bouman et al. 2006; Pommier et al. 2007), but contradicted the hypothesis that a majority of marine bacterioplanktons should be cosmopolitan, endemism should be rare and their global diversity should be low (Fenchel and Finlay 2004), i.e. marine bacterioplanktons should be ubiquitously distributed. Furthermore, two cosmopolitans (belonging to Bacillus described above) which were abundantly present in surface seawater communities were also detected in other marine bacterioplankton communities (Pommier et al. 2007). The finding was consistent with those of Zeng et al. (2014), who suggested that some cosmopolitans belonging to a few bacterial groups that were usually abundant in marine bacterioplankton communities may have similar ecological functions in similar marine environments but at different geographic locations.

CONCLUSION

In this study, 16S rRNA-related technologies were used to reveal microbial diversity of marine bacterioplankton communities in Drake Passage near the Great Wall Station, Antarctic. The results showed that five communities of surface seawaters in Drake Passage consisted mainly of the same species: Gammaproteobacteria, which was in agreement with the investigations on pelagic and benthic ecosystems. At a finer phylogenetic resolution, psychrophilic bacteria, including Bacillus, Aeromonas, Psychrobacter, Pseudomonas and Halomonas, were the most abundant groups. As global marine bacterioplankton communities, a high degree of endemism and conversely few cosmopolitans were detected in surface seawaters communities of Drake Passage and cosmopolitans (Bacillus belonging to psychrophilic bacteria) had the largest population sizes. However, little similarity at the OTU level was detected among locations, and the microbial diversity of surface seawater communities did not correlate significantly with latitude. The environmental factors may be the main determinant of the bacterial community composition in surface waters of Drake Passage and need to be confirmed further.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSLE online.

ACKNOWLEDGEMENTS

This study was supported by China postdoctoral science foundation and postdoctoral application foundation of Qingdao (grant numbers G19201447, Q51201408).

Conflict of interest. None declared.

REFERENCES

- Bouman HA, Ulloa O, Scanlan DJ, et al. Oceanographic basis of the global surface distribution of Prochlorococcus ecotypes. Science 2006;**312**:918–21.
- Che S, Song L, Song W, et al. Complete genome sequence of Antarctic bacterium Psychrobacter sp. strain G. Genome Announc 2013;1:e00725-13.
- Cottrell MT, Kirchman DL. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl Environ Microb 2000a;66:5116-22.
- Cottrell MT, Kirchman DL. Natural assemblages of marine Proteobacteria and members of Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. Appl Environ Microb 2000b;66:1692-7.
- Cristóbal HA, Benito J, Lovrich GA, et al. Phylogenentic and enzymatic characterization of psychrophilic and psychrotolerant marine bacteria belong to γ -Proteobacteria group isolated from the sub-Antarctic Beagle Channel, Argentina. Folia Microbiol (Praha) 2015;60:183-98.
- D'Amico S, Collins T, Marx JC, et al. Psychrophilic microorganisms: challenges for life. EMBO Rep 2006;7:385-9.
- DeSantis TZ, Hugenholtz P, Larsen N, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microb 2006;72:5069-72.
- Dong N, Di Z, Yu Y, et al. Extracellular enzyme activity and antimicrobial activity of culturable bacteria isolated from soil of Grove Mountains, East Antarctica. Wei Sheng Wu Xue Bao 2013;53:1295-306.
- Fenchel T, Finlay BJ. The ubiquitous of small species: patterns of local and global diversity. Bioscience 2004;54:777-84.
- Fuhrman JA, Steele JA, Hewson I, et al. A latitudinal diversity gradient in planktonic marine bacteria. P Natl Acad Sci USA 2008;105:7774-8.
- Ghiglione JF, Galand PE, Pommier T, et al. Pole-to-pole biogeography of surface and deep marine bacterial communities. P Natl Acad Sci USA 2012;109:17633-8.
- Gilbert JA, Hill PJ, Dodd CER, et al. Demonstration of antifreeze protein activity in Antarctic lake bacteria. Microbiology 2004;150:171-80.
- González JM, Fernández-Gómez B, Fernández-Guerra A, et al. Genome analysis of the proteorhodopsin-containing marine bacterium Polaribacter sp. MED152 (Flavobacteria). P Natl Acad Sci USA 2008;105:8724-9.
- Gouvêa Taketani R, Domingues Zucchi T, Soares de Melo I, et al. Whole-genome shotgun sequencing of Rhodococcus erythropolis strain P27, a highly radiation-resistant Actinomycete from Antarctica. Genome Announc 2013;1:e00763-13.
- Halpern BS, Walbridgen S, Selkoe KA, et al. A global map of human impact on marine ecosystems. Science 2008;319:948-52.
- Hillebrand H. On the generality of the latitudinal diversity gradient. Am Nat 2004;163:192-211.
- Huber T, Faulkner G, Hugenholtz P. Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 2004;20:2317-9.
- Jadhav VV, Pote SS, Yadav A, et al. Extracellular cold active lipase from the psychrotrophic Halomonas sp. BRI 8 isolated from the Antarctic sea water. Songklanakarin J Sci Technol 2013;**35**:623–30.
- Jamieson RE, Rogers AD, Billett DS, et al. Patterns of marine bacterioplankton biodiversity in the surface waters of the Scotia Arc, Southern Ocean. FEMS Microbiol Ecol 2012;80: 452-68.

- Juni E, Heym GA. Psychrobacter immobilis gen. nov., sp. nov.: genospecies composed of gram-negative, aerobic, oxidasepositive coccobacilli. Int J Syst Evol Micr 1986;36:388-91.
- Kaye JZ, Márquez MC, Ventosa A, et al. Halomonas neptunia sp. nov., Halomonas sulfidaeris sp. nov., Halomonas axialensis sp. nov. and Halomonas hydrothermalis sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. Int J Syst Evol Micr 2004;54:499-511.
- Kim YO, Heo YL, Kim HK, et al. Gene cloning and characterization of a cold-adapted esterase from Acinetobacter venetianus V28. J Microbiol Biotechn 2012;22:1245-52.
- Maiangwa J, Ali MS, Salleh AB, et al. Adaptational properties and applications of cold-active lipases from psychrophilic bacteria. Extremophiles 2015;19:235-47.
- Manganelli M, Malfatti F, Samo TJ, et al. Major role of microbes in carbon fluxes during austral winter in the Southern Drake Passage. PLoS One 2009;4:e6941.
- Männistö MK, Häggblom MM. Characterization of psychrotolerant heterotrophic bacteria from Finnish Lapland. Syst Appl Microbiol 2006;29:229-43.
- Margesin R, Miteva V. Diversity and ecology of psychrophilic microorganisms. Res Microbiol 2011;162:346-61.
- Mohamad Ali MS, Mohd Fuzi SF, Ganasen M, et al. Structural adaptation of cold-active RTX lipase from Pseudomonas sp. strain AMS8 revealed via homology and molecular dynamics simulation approaches. Biomed Res Int 2013;2013:925373.
- Morita RY. Psychropilic bacteria. Bacteriol Rev 1975;39:144-67.
- Morris RM, Rappé MS, Connon SA, et al. SAR11 clade dominates ocean surface bacterioplankton communities. Nature 2002;420:806-10.
- Murray AE, Grzymski JJ. Diversity and genomics of Antarctic marine micro-organisms. Philos T R Soc B 2007;362:2259-71.
- Murray AE, Preston CM, Massana R, et al. Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl Environ Microb 1998;64:2585-95.
- Muryoi N, Sato M, Knaeko S, et al. Cloning and expression of afpA, a gene encoding an antifreeze protein from the arctic plant growth-promoting rhizobacterium Pseudomonas putida GR12-2. J Bacteriol 2004;186:5661-71.
- Oren A. Halophilic microbial communities and their environments. Curr Opin Biotechnol 2015;33:119-24.
- Pommier T, Canbäck B, Riemann L, et al. Global patterns of diversity and community structure in marine bacterioplankton. Mol Ecol 2007;16:867-80.
- Pope PB, Mackenzie AK, Gregor I, et al. Metagenomics of svalbard reindeer rumen microbiome reveals abundance of polysaccharide utilization loci. PLoS One 2012;7:e38571.
- Rappé MS, Vergin K, Giovannoni SJ. Phylogenetic comparisons of a coastal bacterioplankton community with its counterparts in open ocean and freshwater systems. FEMS Microbiol Ecol 2000;33:219-32.
- Schloss PD, Westcott SL, Ryabin T, et al. Introducing mother: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microb 2009;75:7537-41.
- Sul WJ, Oliver TA, Ducklow HW, et al. Marine bacteria exhibit a bipolar distribution. P Natl Acad Sci USA 2013;110:2342-7.
- Tytgat B, Verleyen E, Obbels D, et al. Bacterial diversity assessment in Antarctic terrestrial and aquatic microbial mats: a comparison between bidirectional pyrosequencing and cultivation. PLoS One 2014;9:e97564.
- Vasileva-Tonkova E, Romanovskaya V, Gladka G, et al. Ecophysiological properties of cultivable heterotrophic bacteria and

- yeasts dominating in phytocenoses of Galindez Island, maritime Antarctica. World J Microb Biot 2014;30:1387-98.
- Waller RG, Scanlon KM, Robinson LF. Cold-water coral distributions in the Drake Passage area from towed camera observations—initial interpretations. PLoS One 2011;6:e16153.
- Wang W, Wang F, Ji X, et al. Cloning and characterization of a psychrophilic catalase gene from an antarctic bacterium. Afr J Microbiol Res 2011;5:3195-9.
- Xing M, Hou Z, Yuan J, et al. Taxonomic and functional metagenomic profiling of gastrointestinal tract microbiome of the farmed adult turbot (Scophthalmus maximus). FEMS Microbiol Ecol 2013;86:432-43.
- Yerqeau E, Schoondermark-Stolk SA, Brodie EL, et al. Environmental microarray analyses of Antarctic soil microbial communities. ISME J 2009;3:340-51.

- Yumoto I, Hirota K, Iwata H, et al. Temperature and nutrient availability control growth rate and fatty acid composition of facultatively psychrophilic Cobetia marina strain L-2. Arch Microbiol 2004;181:345-51.
- Zeng YX, Yu Y, Qiao ZY, et al. Diversity of bacterioplankton in coastal seawaters of Fildes Peninsula, King George Island, Antarctica. Arch Microbiol 2014;196:137-47.
- Zheng X, Chu X, Zhang W, et al. A novel cold-adapted lipase from Acinetobacter sp. XMA-26: gene cloning and characterization. Appl Microbiol Biot 2011;90:971-80.
- Zinger L, Amaral-Zettler LA, Fuhrman JA, et al. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. PLoS One 2011;6:e24570.
- Zubkov MV, Fuchs BM, Tarran GA, et al. Mesoscale distribution of dominant bacterioplankton groups in the northern North Sea in early summer. Aquat Microb Ecol 2002;29:135-44.