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Phylogenetic diversity of culturable bacteria in surface seawater from the Drake Passage, Antarctica*

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Abstract The Drake Passage is located between the Antarctic Peninsula and Tierra del Fuego in the south of South America. Surface seawater samples were collected at seven sites in the Drake Passage during the austral summer of 2012. The 16S rRNA sequences were analyzed from 187 isolated bacterial strains. Three phyla, 29 genera and 56 species were identified. The three phyla were Actinobacteria, Firmicutes and Proteobacteria; the Proteobacteria included α-Proteobacteria, β-Proteobacteria and γ-Proteobacteria. γ-Proteobacteria, Actinobacteria and Firmicutes were the dominant class or phyla in terms of quantity and species. Gram-positive bacteria (Actinobacteria and Firmicutes) accounted for 57.8% of all types identified. There were nine dominant genera, including *Curtobacterium*, *Staphylococcus*, and *Halomonas*, and 14 dominant species including *Curtobacterium flaccumfaciens*, *Curtobacterium pusillum*, and *Staphylococcus sciuri*. Of the strains identified, 87.2% were catalase positive or weakly positive.

Keyword: Antarctic; surface seawater; culturable bacteria; phylogenetic diversity; catalase activities

1 INTRODUCTION

The Drake Passage is located between Tierra del Fuego in the south of South America and the Antarctic Peninsula. During the austral summer of l999/2000, chlorophyll *a* and primary productivity were observed in the surface waters of the area encircling Antarctic. The results showed that the Drake Passage was the most barren of the Antarctic waters, with lower chlorophyll *a* and primary productivity than in other areas (Liu et al., 2000). In addition, an earlier survey found that the particulate organic carbon (POC) content in the Drake Passage was also lower than in most of the other ocean areas around Antarctica (Liu et al., 1993).

The formation of hydrogen peroxide (H_2O_2) in temperate surface waters is primarily driven by UV-induced photochemical activation of dissolved organic carbon (DOC) (Cooper and Zika, 1983; Moffett and Zajiriou, 1990; Karl et al., 1993; Abele et al., 1999). Hydrogen peroxide is a cytotoxic reactive oxygen species, which can elicit oxidative stress in marine organisms (Viarengo et al., 1998). H_2O_2 elicits oxidative damage of the membrane lipids (Thomas and Reed, 1990; Stark, 1991), proteins (Neužil et al., 1993) and nucleic acids (Schulte-Frohlinde and Sonntag, 1985) via the liberation of highly reactive hydroxyl radicals inside the cells. Thereby, it can interfere with membrane transport (Jones, 1985; Hitschke et al., 1994; Mense et al., 1997) and generally disturb cellular homeostasis (Boraso and Williams, 1994; Abele-Oeschger et al., 1996; Abele et al., 1998; Abele et al., 1999).

Marine bacterioplankton communities in temperate and Polar regions affect global energy, atmospheric-oceanic interactions, and the oceanic food web (Legendre et al., 1992; Brown and Bowman, 2001;

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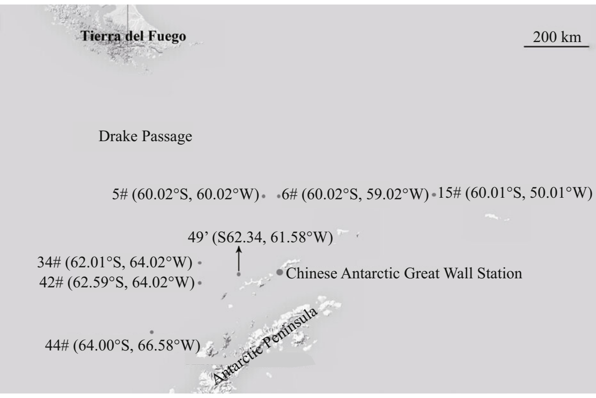

Fig.1 Sampling sites in the Drake Passage in Antarctica

Prabagaran et al., 2007). Bacterioplankton community structure can therefore be used as an indicator of marine ecosystem status (Zeng et al., 2014). Although molecular biological techniques, such as 16S rRNA gene sequencing and metagenomics, have been widely used in microbial community studies, the drawback of these studies is that DNA sequence data can raely infer physiology (Sievert et al., 1999). Therefore, a pure culture method is essential for studying the physiology of microorganisms and the biogeochemical processes occurring in marine environments.

The aim of this study was to survey the phylogenetic diversity of culturable bacteria in surface seawater samples from seven sites in the Drake Passage in Antarctica, and assess the ability of these bacterial strains to detect catalase activities.

2 MATERIAL AND METHOD

2.1 Sample description and handling

Surface seawater samples were collected from seven sites (Fig.1) in the Drake Passage in Antarctica during the austral summer of 2012. Sites 44, 34, 5 and 15 were at the edge of the sampling range, site 49 was at the center of the sampling range, and sites 6 and 42 were near the edge and the center, respectively. Seawater samples were pressure filtered through 0.2-μm polycarbonate membrane filters (Whatman). The filters were placed in sterile 2-mL centrifuge tubes and covered with 1.0 mL glycerol. After processing, the tubes were immediately frozen and stored at -80°C before further examination.

2.2 Bacterial isolation

Each filter was diluted into sterile artificial seawater (ASW) and then inoculated onto Marine Agar (Difco, MA), Marine R2A Agar (Fluka R2A agar was prepared with seawater instead of distilled water, R2A) and Seawater Agar (seawater and agar only, Difco, pH 7.5, SA). Every plate was incubated at 4°C, 16°C or 28°C, until bacterial colonies could be observed. Individual colonies were selected and purified by streaking three times on fresh medium. Stocks were preserved at -80°C in sterile 0.9% (w/v) NaCl supplemented with 15% (v/v) glycerol.

2.3 DNA extraction and PCR amplification of the 16S rRNA gene

Genomic DNA of the isolates was extracted by phenol/chloroform extraction, with lysozyme added in advance. To determine the phylogenetic characteristics, 16S rRNA genes were amplified and sequenced. The 16S rRNA bacterial genes were amplified by PCR using bacterial primers 27F: 5′-GAGTTTGATCCTGGCTCAG-3′ and 1492R: 5′-CGGCTACCTTGTTACGAC-3′ (Weisburg et al., 1991). PCR products of the 16S rRNA gene were purified, and sequencing reactions were carried out using ABI BigDye 3.1 Sequencing Kit (Applied Biosystems) and an automated DNA sequencer (model ABI3730; Applied Biosystems). In some strains, there were multi-copy 16S rRNA genes, so cloning experiments were conducted before sequencing. Purified PCR products were ligated to the pMD 18-T (TaKaRa, Dalian) and cloned to *Escherichia coli* JM109, according to the manufacturer's instructions.

2.4 Phylogenetic and statistical analyses

The near-complete 16S rRNA gene sequences of the strains were submitted to GenBank/EMBL to search for similar sequences using the BLAST algorithm. The identification of phylogenetic neighbors and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using NCBI BLAST or EzTaxon server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). Phylogenetic trees were constructed using the neighbor-joining methods with Kimura 2-state parameter model analyses implemented in the program MEGA version 5 (Tamura et al., 2007). In each case, bootstrap values were calculated based on 1 000 replicates. All pie charts and histograms were made using Microsoft Office Excel 97 or 2003. The β-diversity was depicted in a Venn diagram (Oliveros, 2007; http://bioinfogp.cnb.csic.es/tools/venny/index.html).

2.5 Detection of catalase activities

Catalase activity detection was examined in all bacterial isolates. Standard protocols (Tindall et al., 2007) were used to assess catalase activities. The growth was scraped from a plate using a non-metallic instrument, and suspended in a drop of 3% hydrogen peroxide on a slide. The slide was examined for bubbles immediately and again after 5 min, either macroscopically or with a hand lens. If catalase activity was weak, a coverslip was placed over the wet-mount preparation to capture the bubbles.

3 RESULT

3.1 Bacterial isolation

In the seven surface seawater samples from the Drake Passage in Antarctica, 187 bacterial strains were detected, including 74 strains cultured from MA, 85 strains cultured from R2A and 28 strains cultured from SA. The incubation time ranged from 2 to 7 days. GenBank accession numbers were KP296187–KP296242 and KT159326–KT159456.

3.2 Phylogenetic and statistical analyses

The 187 bacterial strains identified included 3 phyla, 29 genera and 56 species. The three phyla were Actinobacteria, Firmicutes and Proteobacteria, with the latter including α-Proteobacteria, β-Proteobacteria and γ-Proteobacteria. There were 7 genera, 11 species and 57 strains belonging to Actinobacteria; 7 genera, 19 species and 51 strains belonging to Firmicutes; 3 genera, 4 species and 4 strains belonging to α-Proteobacteria; 5 genera, 6 species and 14 strains belonging to β-Proteobacteria; and 7 genera, 16 species and 61 strains belonging to γ-Proteobacteria. The taxonomic positions of the 187 strains are shown in Table 1. Figure 2 shows the neighbor-joining tree, depicting the relationships of the three phyla and the details of the "Proteobacteria". Figure 3 shows the neighbor-joining tree of the relationships within the Actinobacteria. Figure 4 shows the neighbor-joining tree of the relationship within the Firmicutes.

Table 1 Taxonomic position and catalase activities of strains isolated from the surface seawater of the Drake Passage in Antarctica

Species	Numbers of strains							Catalase activities		
	44#	34#	5#	6#	15#	49#	42#	+	W	-
Actinobacteria								26	19	12
Curtobacterium flaccumfaciens	1	8	5	10	4	3	4	12	13	10
Curtobacterium pusillum			2	2	5		2	5	5	1
Curtobacterium ammoniigenes							1	1		
Curtobacterium oceanosedimentum						1		1		
Nesterenkonia flava			1						1	
Brachybacterium faecium				1				1		
Microbacterium aoyamense		1						1		
Propionibacterium acnes	2	1						2		
Micrococcus yunnanensis	1							1		
Arthrobacter nicotianae	1									1
Arthrobacter tumbae						1		1		
Firmicutes								27	20	4
Staphylococcus equorum			1	1	4	1	2	4	5	
Staphylococcus saprophyticus			1		3	2	1	1	6	
Staphylococcus cohnii							1	1		
Staphylococcus vitulinus				2				1	1	
Staphylococcus sciuri		8			1		2	5	5	1
Bacillus stratosphericus							1	1		
Bacillus licheniformis	3	3	1				1	7		1
Bacillus isronensis	1							1		

To be continued

Table 1 Continued

Species	Numbers of strains							Catalase activities		
	$44^{\#}$	$34^{\#}$	$5^{\#}$	$6^{\#}$	$15^{\#}$	$49^{\#}$	$42^{\#}$	+	W	-
Bacillus sonorensis			1						1	
Bacillus axarquiensis		1						1		
Bacillus subtilis		1							1	
Bacillus atrophaeus		1								1
Bacillus cereus	1							1		
Bacillus mojavensis	1							1		
Jeotgalicoccus huakuii			1							1
Planomicrobium okeanokoites				1				1		
Exiguobacterium profundum			1						1	
Ornithinibacillus contaminans		1						1		
Oceanobacillus oncorhynchi						1		1		
α-Proteobacteria									4	
Gemmobacter changlensis						1			1	
Sphingomonas echinoides					1				1	
Paracoccus marcusii							1		1	
Paracoccus tibetensis							1		1	
β-Proteobacteria								7	5	2
Methyloversatilis thermotolerans					1			1		
Methyloversatilis universalis			1		2	1			3	1
Limnobacter thiooxidans					4		2	4	1	1
Methylophilus flavus							1		1	
Ralstonia insidiosa							1	1		
Cupriavidus gilardii		1						1		
γ-Proteobacteria								36	19	6
Halomonas hamiltonii					3	1	6	3	5	2
Halomonas stevensii	3		3		1	1	1	4	4	1
Halomonas axialensis							1	1		
Halomonas meridiana	1	2		2			1	5		1
Halomonas sulfidaeris						1		1		
Acinetobacter oryzae							1	1		
Acinetobacter lwoffii				3	4	1	2	5	4	1
Acinetobacter johnsonii							1		1	
Aeromonas punctata					1				1	
Aeromonas hydrophila	1							1		
Salinicola salarius				1				1		
Escherichia albertii	8	1		1				10		
Vibrio alginolyticus						2			1	
Vibrio fortis						5		3	2	
Vibrio algoinfesta						1		1		1
Stenotrophomonas maltophilia					1			1	1	
Total	**24**	**29**	**18**	**24**	**35**	**23**	**34**	**96**	**67**	**24**

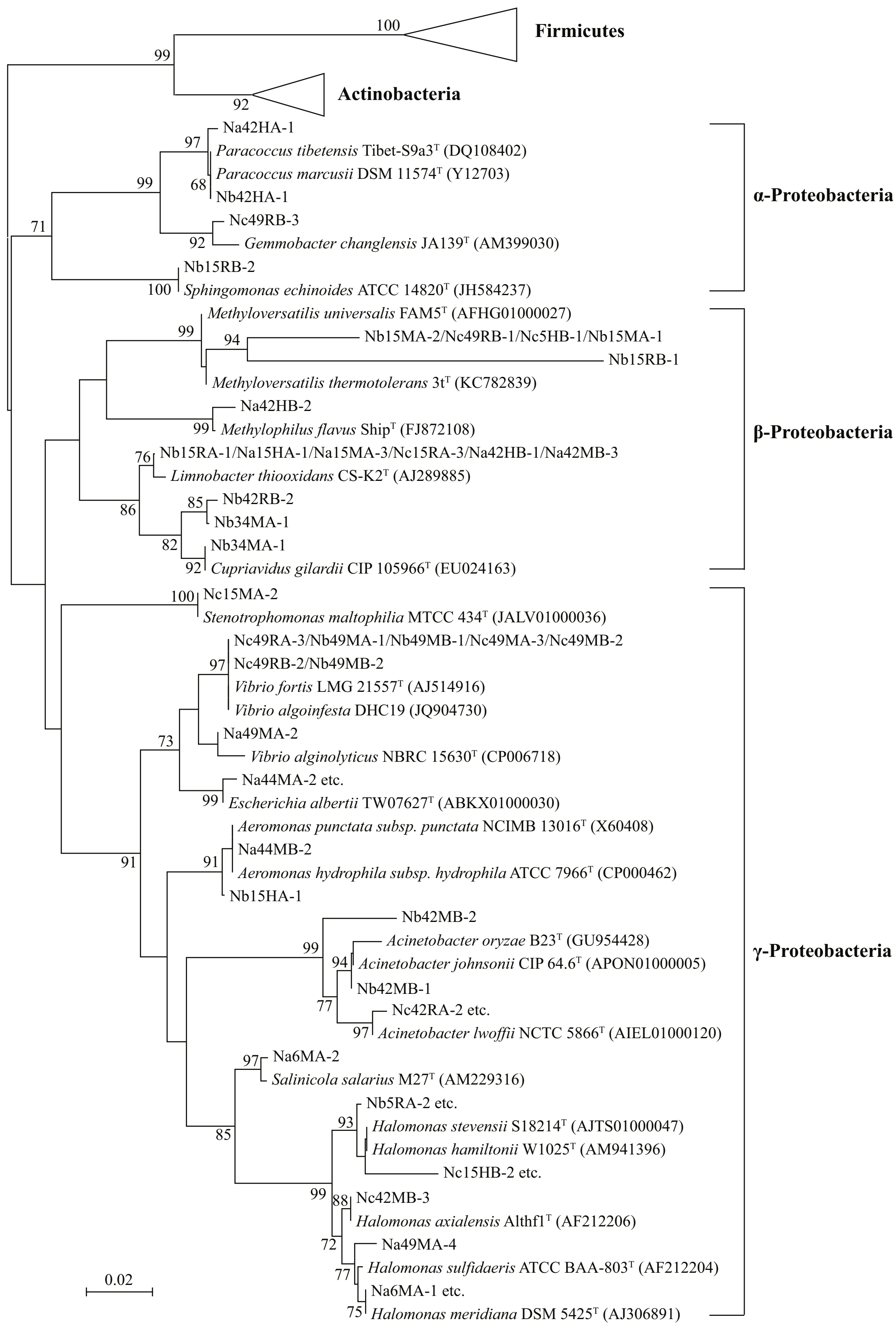

Fig.2 Neighbor-Joining tree showing the relationship of the three phyla and the details of "Proteobacteria"

Na44MA-2 etc.: Na44MA-2/ Nb34RA-4/ Nc6MB-1/ Na44RA-1/ Na44RA-3/ Na44RB-1/ Na44RB-2/ Na44MB-1/ Nb44RA-1/ Nb44RB-1; Nc42RA-2 etc.: Nc42RA-2/ Nc49HA-1/ Nc15MA-1/ Nb15HA-2/ Nc15HA-1/ Nc15HA-2/ Nc42RB-1/ Na6HB-1/ Na6MB-1/ Nb6RA-2; Nb5RA-2 etc.: Nb5RA-2/ Nc49RA-1/ Nc5MA-4/ Nc15HB-1/ Nc42RB-2/ Na44RB-3/ Na44MA-1/ Nb44MA-1/ Na5RA-1; Nc15HB-2 etc.: Nc15HB-2/ Nc49MA-2/ Nb15HB-2/ Nc15RA-4/ Na42RB-1/ Na42MA-1/ Nb42RA-1/ Nb42RB-1/ Na42HA-2/ Nb42HB-2; Na6MA-1 etc.: Na6MA-1/ Na34MA-3/ Nc6RB-2/ Nb34MA-3/ Na42MB-3/ Nc44RA-2.

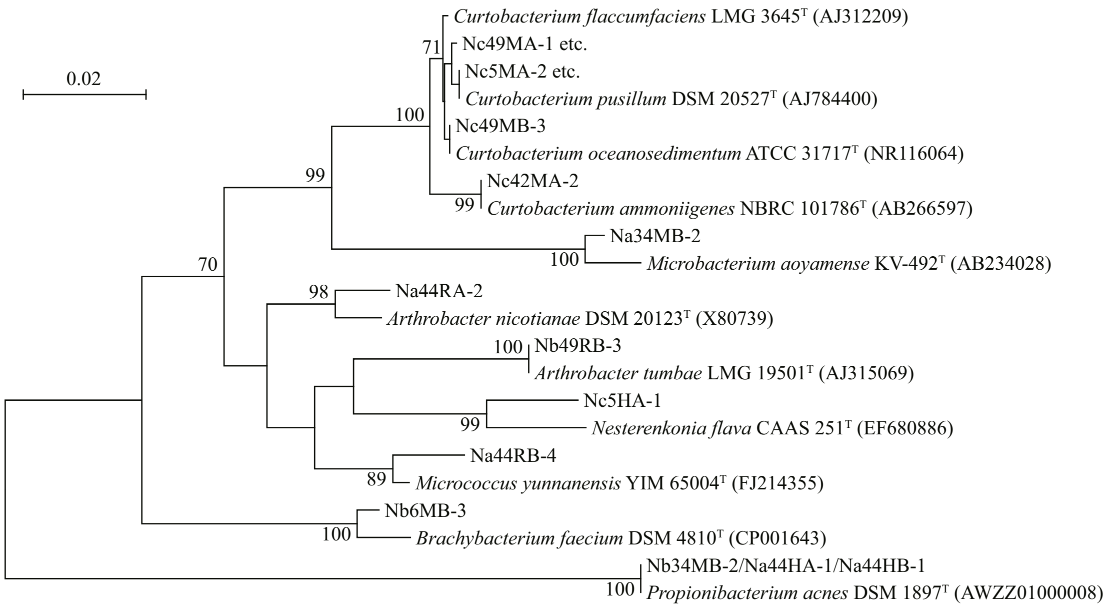

Fig.3 Neighbor-Joining tree showing the relationships of Actinobacteria

Nc5MA-2 etc.: Nc5MA-2/ Nc5MB-3N/ Na15RA-2/ Na15RB-2/ Na15MB-1/ Nb15MA-3/ Nb15MB-3/ Na42RA-1/ Nc42RB-3/ Na6HA-1/ Nb6RB-1; Nc49MA-1 etc.: Nc49MA-1/ Na49HA-1/ Nb5RB-2/ Na15RA-1/ Na15MB-4/ Na15HB-1/ Nb15HB-1/ Nb42MA-1/ Nc42HA-1/ Nc42RA-1/ Nc42RA-3/ Na6RB-1/ Nb6HA-1/ Nb6RB-2/ Nb6MA-2/ Nb6MA-3/ Nb6MB-1/ Nb6MB-2/ Nc6RA-1/ Nc6RA-2/ Nc6RB-1/ Nb49RB-1/ Na34RA-5/ Nb34RA-2/ Nb34RA-3/ Nb34RB-2/ Nc34RB-3/ Nb5RA-1/ Nb5RB-1/ Nc5RB-1/ Nc5RB-2/ Na34RA-2/ Na34RA-4/ Nc34RA-2/ Nc44MA-2.

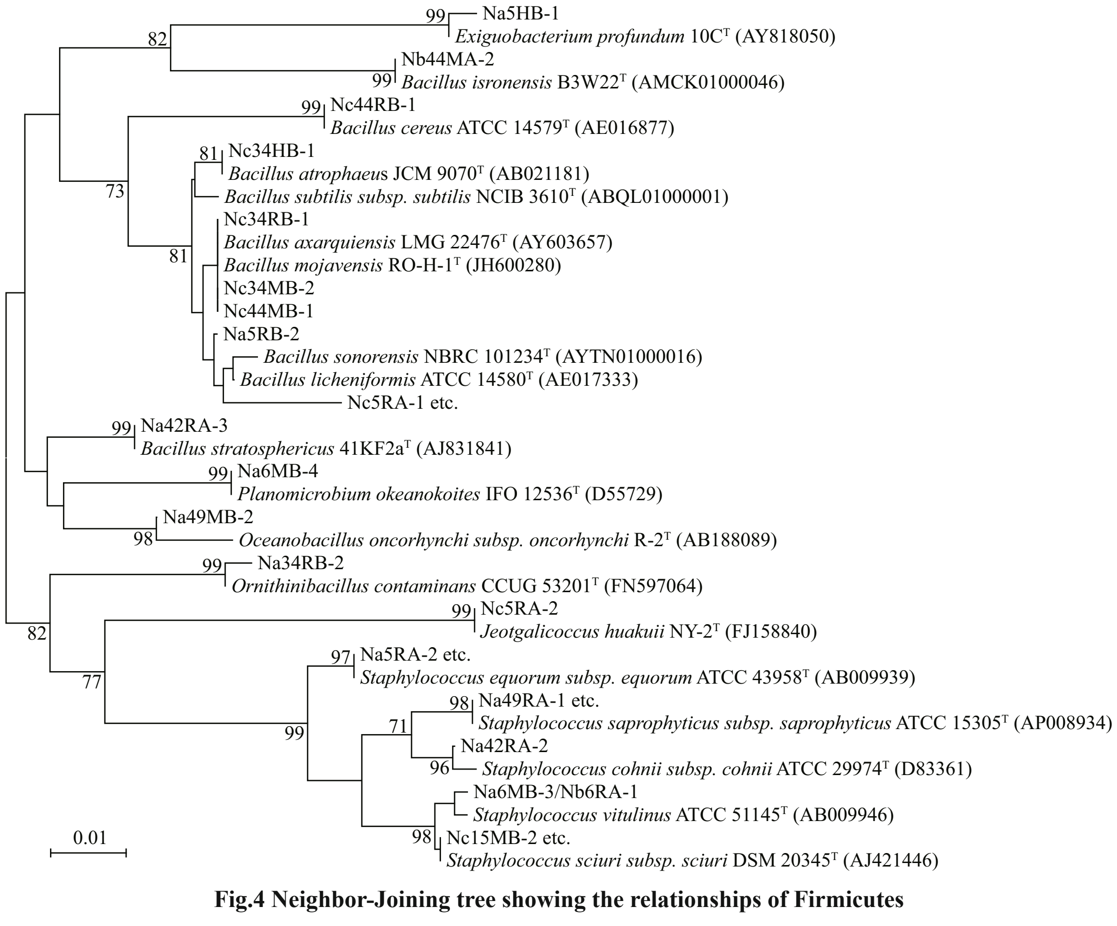

Fig.4 Neighbor-Joining tree showing the relationships of Firmicutes

Nc5RA-1 etc.: Nc5RA-1/ Na42RB-3/ Nc34MA-1/ Nc34MB-1/ Nc34MB-3/ Nc44RB-3/ Nc44MA-3/ Nc44MB-2; Na49RA-1 etc.: Na49RA-1/ Nb49RA-2/ Nc5RA-3/ Na15RA-3/ Na15MB-3/ Nb15MB-2/ Na42RB-2; Nc15MB-2 etc.: Nc15MB-2/ Nb42MA-2/ Nc42MB-1/ Na34RA-1/ Na34RA-3/ Na34RB-3/ Na34RB-4/ Na34MA-1/ Na34MA-2/ Na34MB-1/ Nb34RA-1.

Table 2 Strains composition of culturable bacteria from seven sites

Phylum		Numbers of strains							Total
		44#	34#	5#	6#	15#	49#	42#	
Actinobacteria		5	10	8	13	9	5	7	57
Firmicutes		6	15	6	4	8	4	8	51
Proteobacteria	α-Proteobacteria	0	0	0	0	1	1	2	4
	β- Proteobacteria	0	1	1	0	7	1	4	14
	γ- Proteobacteria	13	3	3	7	10	12	13	61
Total		24	29	18	24	35	23	34	187

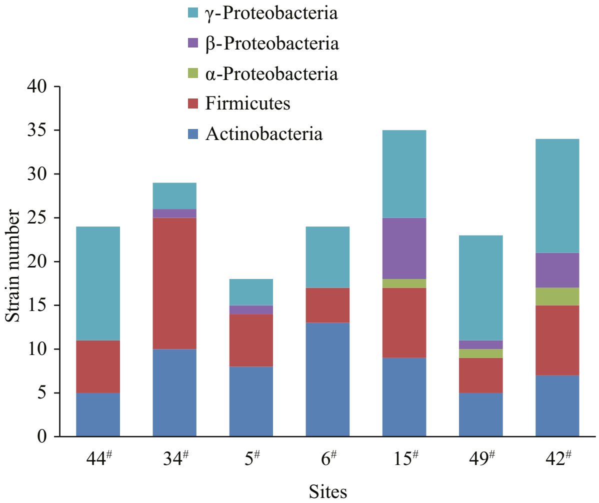

Fig.5 Numbers of different bacterial strains identified at each site

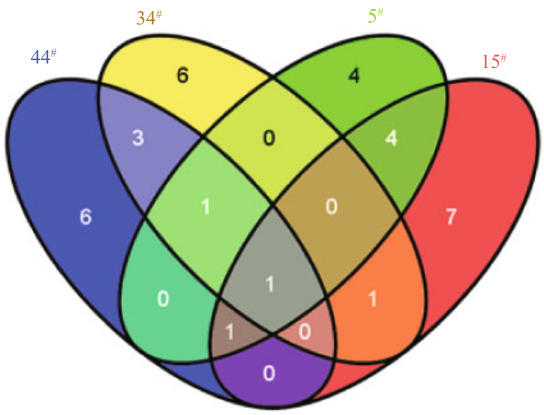

Fig.6 β-diversity between sites far away from land

3.3 Dominant bacterial community

γ-Proteobacteria, Actinobacteria and Firmicutes were the dominant class or phyla in terms of quantity and species. Gram-positive bacteria (Actinobacteria and Firmicutes) accounted for 57.8% of all types identified. There were nine dominant genera, which accounted for 87% of all types: *Curtobacterium*, *Staphylococcus*, *Halomonas*, *Bacillus*, *Acinetobacter*, *Escherichia*, *Vibrio*, *Limnobacter* and *Methyloversatilis*. The most dominant genus was *Curtobacterium*, accounting for 26% of all types. There were 14 dominant species, which accounted for 75% of all types: *Curtobacterium flaccumfaciens*, *Curtobacterium pusillum*, *Staphylococcus sciuri*, *Halomonas hamiltonii*, *Acinetobacter lwoffii*, *Escherichia albertii*, *Halomonas stevensii*, *Staphylococcus equorum*, *Bacillus licheniformis*, *Staphylococcus saprophyticus*, *Limnobacter thiooxidans*, *Halomonas meridiana*, *Vibrio fortis* and *Methyloversatilis universalis*. The most dominant species was *Curtobacterium flaccumfaciens*, accounting for 19% of all types identified.

3.4 Diversity of the culturable bacteria in different sites

The compositions of the strains of culturable bacteria identified at the seven sites are shown in Table 2 and Fig.5. There were 3 phyla, 8 genera, 12 species and 24 strains cultured from site 44; 3 phyla, 9 genera, 12 species and 29 strains cultured from site 34; 3 phyla, 8 genera, 11 species and 18 strains cultured from site 5; 3 phyla, 8 genera, 10 species and 24 strains cultured from site 6; 3 phyla, 9 genera, 14 species and 35 strains cultured from site 15; 3 phyla, 9 genera, 15 species and 23 strains cultured from site 49; and 3 phyla, 9 genera, 21 species and 34 strains cultured from site 42. Figure 6 shows the β-diversity between the sites far away from land (44, 34, 5 and 15). Figure 7 shows the β-diversity between the sites both far away from land and close to land (the strains cultured from the sites 44, 34, 5, 6 and 15 were combined, as were those from sites 49 and 42 together, which were then compared using β-diversity).

3.5 Decomposition of hydrogen peroxide

All the 187 bacterial strains detected the ability to

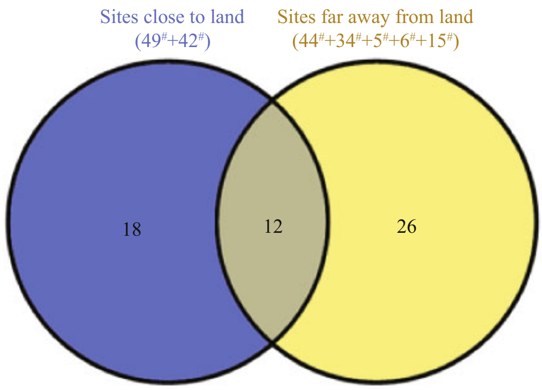

Fig.7 β-diversity compared between sites far away and close to land

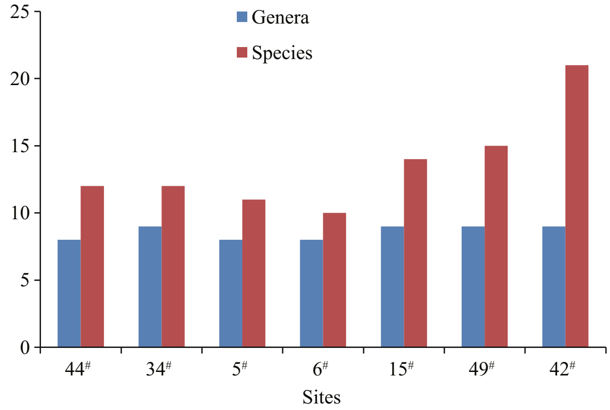

Fig.8 Diversity of bacteria genera and species from each site

decompose hydrogen peroxide (H_2O_2). Of the strains identified, 96 were catalase positive, 67 were weakly catalase positive, and 24 were catalase negative. Of the species identified, 53 of 56 had the ability to decompose H_2O_2. The statistical analysis of catalase activities is shown in Table 1.

4 DISCUSSION

4.1 Comparison of different media

There were 35 species and 74 strains cultured from MA, 30 species and 85 strains cultured from R2A, and 15 species and 28 strains cultured from SA. In both quality and quantity, strains cultured from MA and R2A were similar, but were much higher than those from SA. This demonstrates that MA and R2A were more suitable for culturing marine bacteria. However, it did not mean Seawater Agar was useless, because seven species could be cultured only in SA. This demonstrates that although MA or R2A are preferable, diversified media are necessary as supplements.

4.2 Comparison of different sites

There were five groups cultured from sites 49, 42 and 15, but only three from sites 44, 5 and 6 (Fig.5). The diversity of sites 49 and 42 was higher than 5 and 6, at both the genera and species level (Fig.8). In coastal areas, large rivers and human activities bring in significant sediment loads and dissolved organic carbon, which modifies surface waters. In these areas, many terrestrial bacteria are being taken into the ocean, and so bacterial communities are gradually changing (Brinkmeyer et al., 2003; Ghiglione et al., 2012). Compared the results above with Fig.1, it could be seen that the diversity of sites close to the land was higher than sites far away from the land. This was probably due to the terrestrial influence on seawater bacterial communities, and thus the ease of culturing bacteria in seawater from close to the land. On the contrary, as the terrestrial influence on sites far away from land was smaller, and authentic marine bacteria were difficult to culture, the diversity of microbes far away from the land was lower. In conclusion, it is important to improve the techniques for culturing different bacteria.

4.3 Comparison with other localities of the Antarctic and Arctic

4.3.1 Comparison with culture-dependent studies in the Antarctic and Arctic

Before the 21st century, studies of microbes in Antarctic (including Drake Passage) water or ice focused on the microbial biomass. In 2003, 78 pure cultured bacteria were isolated from Antarctic pack ice, and all the strains were gram-negative (Brinkmeyer et al., 2003). Prior to 2006, no gram-positive bacteria were cultured from the Arctic ice (Brinkmeyer et al., 2003; Yu et al., 2006). More recently, gram-positive bacteria have been cultured in the following studies of Antarctic (De Souza et al., 2006; Antony et al., 2012; Zdanowski et al., 2013) and Arctic (Groudieva et al., 2004; Zeng et al., 2012) water or ice, though the proportions were small. This study was the first to find both Actinobacteria and Firmicutes as dominant phyla in terms of quantity and species in culture-dependent studies, and gram-positive bacteria (Actinobacteria and Firmicutes) accounted for more than 50% of the strains identified. At the genus level, 29 genera were cultured in this study, but only 5–10 genera were cultured in the aforementioned studies from Antarctic seawater or sea ice, and only 9–16 genera were cultured in the aforementioned studies from Arctic seawater or sea ice. In this study, 10 of the 29 genera were also found in the aforementioned studies: *Brachybacterium*, *Micrococcus*, *Arthrobacter*, *Bacillus*, *Planomicrobium*, *Sphingomonas*, *Halomonas*, *Acinetobacter*, *Aeromonas* and *Vibrio*. Of the 56 species, 37 have been found in the marine environment (seawater, hydrothermal vent, deep-sea sediment, mangrove sediment, marine organism and so on) (searched from NCBI), which means that the other 19 species were cultured from the marine environment for the first time. The proposed reasons for the high proportion of Gram-positive bacteria and the high diversity at genus and species level in this study are as follows: (1) phylogenetic surveys of the world's oceans and lakes (Britschgi and Giovannoni, 1991; Fuhrman et al., 1993; Mullins et al., 1995; Zwart et al., 1998; Glöckner et al., 2000; Bano and Hollibaugh, 2002; Hollibaugh et al., 2002) have strongly suggested mixing of bacterial populations on a global scale (Brinkmeyer et al., 2003; Gupta et al., 2015). Increasing human activities have resulted in increased terrestrial influence on the Southern Ocean. Previous studies have suggested that at least some members of the typical soil or freshwater groups (Betaproteobacteria, Verrucomicrobia, and Actinobacteria) are able to survive and proliferate in marine environments (Riemann et al., 2008; Zeng et al., 2009, 2012); (2) there were three culture temperatures (4°C, 16°C and 28°C) and three kinds of medium suitable for marine bacteria were used in this study to obtain more strains; (3) the samples in the aforementioned studies were mostly sea ice or seawater collected during the austral winter; many bacteria may have been "viable but nonculturable" (Xu et al., 1982), or in the form of spores. In this study, seawater samples were collected during the austral summer, so the bacteria were more likely to survive and it was easier to germinate spores in the lab. In this study, several genera were reported that may have been viable but nonculturable, such as *Aeromonas*, *Arthrobacter*, *Escherichia*, *Micrococcus* and *Vibrio* (Zhang, 2007).

Previously, gram-negative bacteria were considered to be dominant in seawater (Seiburth, 1979), and gram-positive bacteria were widely distributed in deep-sea sediments (Bowman et al., 2000; Ravencehlag et al., 2001) and marine organisms' symbiotic systems (Webster et al., 2001). However, some studies have reported the recovery of more non-spore forming bacteria from ice, especially in the deeper sections. It was thus suggested that these bacteria were able to survive and maintain themselves for extended periods in cold and desiccated conditions such as in ice (Antony et al., 2012). This may be because DNA in non-spore forming bacteria degrades far more slowly than that in endospore formers because of the maintenance of metabolic activity and DNA repair in the former (Johnson et al., 2007). Considering the results of this study, the conclusions above are not absolute, and more studies with a variety of bacteria detection methods are needed to understand the bacterial composition of the marine environment.

4.3.2 Comparison with culture-independent studies in the coastal areas near the Drake Passage

Zeng employed 454 pyrosequencing of the 16S rRNA gene to obtain a snapshot of microbial community structure in the coastal waters of King George Island, Antarctica (Zeng et al., 2014), which is near the Drake Passage. Less than 10% of the bacterial strains identified were gram-positive in the coastal waters of King George Island; however, gram-positive bacteria (Actinobacteria and Firmicutes) in this study accounted for 57.8%. This was probably for the following reasons: (1) gram-positive bacteria usually exist in the form of spores in extreme environments, such as the seawater of Antarctica. The spores will germinate under appropriate conditions in the laboratory during culturing. However, it is difficult to extract DNA from spores, particularly with 454 pyrosequencing; (2) the cell wall of gram-positive bacteria is thicker and more complex than gram-negative bacteria, so it is difficult to extract DNA from gram-positive bacteria from environmental samples. However, once pure cultures of gram-positive bacteria have been obtained, many methods can be used to extract the DNA, such as adding lysozyme and choosing specific PCR primer. Therefore, pure culture methods can not only enhance the understanding of the bacteria community, but also increase the chance of finding new compounds, because marine actinomycetes were found to be important contributors to search and discovery for novel natural products (Ward and Bora, 2006). Most sequences from the coastal waters of King George Island were only assigned to phylum level. This was probably because the sequences from high-throughput

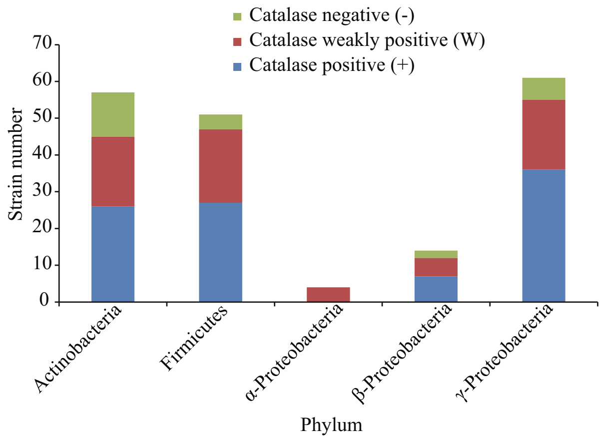

Fig.9 Comparison of catalase activities in different phyla

sequencing were too short to assign to genus level, so it was not valid to compare the two studies at genus level. Although the 454 pyrosequencing could not give as much detail at the species or even genus as the culturing method, Bacteroidetes, δ-Proteobacteria and ε-Proteobacteria were identified in the coastal waters of King George Island, in addition to the phyla mentioned in this study. Therefore, high-throughput sequencing provided some important additional information to the culturing method, especially at the phylum level.

4.4 Decomposition of hydrogen peroxide

The ozone layer or ozone shield refers to a region of the Earth's stratosphere that absorbs most of the Sun's ultraviolet (UV) radiation, with the ozone hole resulting in high UV radiation in the Antarctic. The formation of hydrogen peroxide (H_2O_2) in temperate surface seawater is primarily driven by UV-induced photochemical activation of dissolved organic carbon (DOC) (Cooper and Zika 1983; Moffett and Zajiriou 1990; Karl et al., 1993; Abele et al., 1999). Marine microorganisms could be damaged by H_2O_2. It was therefore speculated that microorganisms in the surface seawater of Antarctica could secrete catalase to protect themselves from H_2O_2 damage. In this study, 87.2% of strains were catalase positive or weakly positive, while 30.5% of strains could decompose H_2O_2 strongly. Of the 56 species, 53 had the ability to decompose H_2O_2, which confirm the aforementioned speculation. There were no significant differences between different sites in the study, probably because high UV radiation is a widespread phenomenon in Antarctica. The γ-Proteobacteria strains had the greatest ability to decompose H_2O_2 (Fig.9), with Firmicutes and Actinobacteria strains not far behind. Strains of these phyla could efficiently dispel the damage from H_2O_2, which is likely to be one of the reasons why γ-Proteobacteria, Actinobacteria and Firmicutes were the dominant classes or phyla.

5 CONCLUSION

Of the 187 bacterial strains cultured from the seven surface seawater samples of the Drake Passage in Antarctica, 3 phyla, 29 genera and 56 species were identified. The three phyla were Actinobacteria, Firmicutes and Proteobacteria, with the latter including α-Proteobacteria, β-Proteobacteria and γ-Proteobacteria. This was the first study to find both Actinobacteria and Firmicutes as dominant phyla in terms of quantity and species in the surface seawater of the Antarctic in culture-dependent studies. Gram-positive bacteria (Actinobacteria and Firmicutes) accounted for more than 50% of the types identified in this study. The bacterial diversity was higher than other studies at both the genus and species level. The diversity at sites close to land was higher than sites far away from land. There were 19 species cultured from the marine environment for the first time. 87.2% strains were catalase positive or weakly positive, and 30.5% strains could decompose H_2O_2 strongly. Of the 56 species identified, 53 had the ability to decompose H_2O_2.

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