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ORIGINAL PAPER

Diversity of bacterioplankton in coastal seawaters of Fildes Peninsula, King George Island, Antarctica

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Abstract The bacterioplankton not only serves critical functions in marine nutrient cycles, but can also serve as indicators of the marine environment. The compositions of bacterial communities in the surface seawater of Ardley Cove and Great Wall Cove were analyzed using a 16S rRNA multiplex 454 pyrosequencing approach. Similar patterns of bacterial composition were found between the two coves, in which Bacteroidetes, Alphaproteobacteria, and Gammaproteobacteria were the dominant members of the bacterioplankton communities. In addition, a large fraction of the bacterial sequence reads (on average 5.3 % per station) could not be assigned below the domain level. Compared with Ardley Cove, Great Wall Cove showed higher chlorophyll and particulate organic carbon concentrations and exhibited relatively lower bacterial richness and diversity. Inferred metabolisms of summer bacterioplankton in the two coves were characterized by chemoheterotrophy and photoheterotrophy. Results suggest that some cosmopolitan species (e.g., *Polaribacter* and *Sulfitobacter*) belonging to a few bacterial groups that usually dominate in marine bacterioplankton communities may have similar ecological functions in similar marine environments but at different geographic locations.

Keywords Marine bacterioplankton · Diversity · Pyrosequencing · King George Island

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Introduction

Marine microorganisms represent the main form of biomass in oceans, and bacterial cell numbers in the upper water layer are typically 10^8–10^9 cells L^{-1} (Ducklow 2000; Granéli et al. 2004). Marine bacterioplankton communities from temperate and polar regions affect global energy, atmospheric–oceanic interactions, and the oceanic food web (Legendre et al. 1992; Brown and Bowman 2001; Prabagaran et al. 2007). Bacterioplankton community structure also can be used as an indicator of marine ecosystem status. Crude oil-induced structure shift of marine bacterioplankton communities has been observed in cold regions (Yakimov et al. 2004; Prabagaran et al. 2007). An important step toward understanding the functions of various bacteria in the ocean is the determination of the numbers and relative abundances of different bacterial groups (Cottrell and Kirchman 2000a; Giovannoni and Rappé 2000).

Culture-independent studies are essential to determine the biodiversity of marine bacterial communities, because only a small fraction of naturally occurring bacterial assemblages can be cultured using currently available methods (Amann et al. 1995). New technologies, such as 454 pyrosequencing, have recently become increasingly popular among microbiologists investigating microbial community structure in marine environments (Bowman et al. 2012; Zeng et al. 2013). This approach is powerful for examining all aspects of microbial

diversity, including rare microbes, thereby providing deep insights into the structure of microbial communities (Sogin et al. 2006; Huber et al. 2007). The dominance of the phylum Bacteroidetes, classes Alphaproteobacteria and Gammaproteobacteria, as well as the genera *Polaribacter*, *Sulfitobacter*, and *Loktanella* has been reported in coastal Antarctic marine bacterioplankton communities (Ghiglione et al. 2012; Ghiglione and Murray 2012; Jamieson et al. 2012).

Ardley Cove lies north of Ardley Island in Maxwell Bay, King George Island, Antarctica. The cove is located at the proximity of the Russian Bellingshausen Station and the Chilean Frei Station. Lying southwest of Ardley Island, Great Wall Cove is located to the east of the Chinese Great Wall Station. Seawater exchange exists between the two coves when high tide occurs, indicating that it appears to exist a mixing between the microbial communities in the two coves. In austral summer, freshwater input from rain, snow, and melting ice is evident in this area. Abundant bacteria (10^7–10^8 cells L^{-1}) have been detected in the coastal seawaters (Ilinskiy and Gorshkov 2004). However, information concerning the biodiversity of the bacterioplankton community in this area remains insufficient. This study aims to facilitate understanding of the species diversity and environmental complexity of these Antarctic coastal microbial mats and to provide insights into the presence of cosmopolitan species that may be important in biochemical processes. We 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.

Materials and methods

Field measurements, sample collection, and bacterial counts

Field measurements and sample collection were conducted in December 2011 during the 28th Chinese National Antarctic Research Expedition. Surface water samples were collected from Ardley Cove and Great Wall Cove (Fig. 1). The location, sample dates, and biogeochemical properties of the samples analyzed in this study are summarized in Table 1. Water temperature and salinity were measured using an YSI Model 30 (Yellow Springs Instruments, Yellow Springs, USA). Nutrients, including nitrate (NO_3^-), nitrite (NO_2^-), silicate (SiO_3^{2-}), and phosphate (PO_4^{3-}), were measured spectrophotometrically with a continuous flow autoanalyzer Scan^{++} (Skalar, the Netherlands) after filtering seawater through 0.45-μm cellulose acetate membrane filters (Whatman) as described by Hansen and Koroleff (1999). Dissolved oxygen (DO) concentration was determined by the Winkler titration method (Strickland and Parsons 1972). Particulate organic carbon (POC) was collected by filtration through combusted glass–fiber

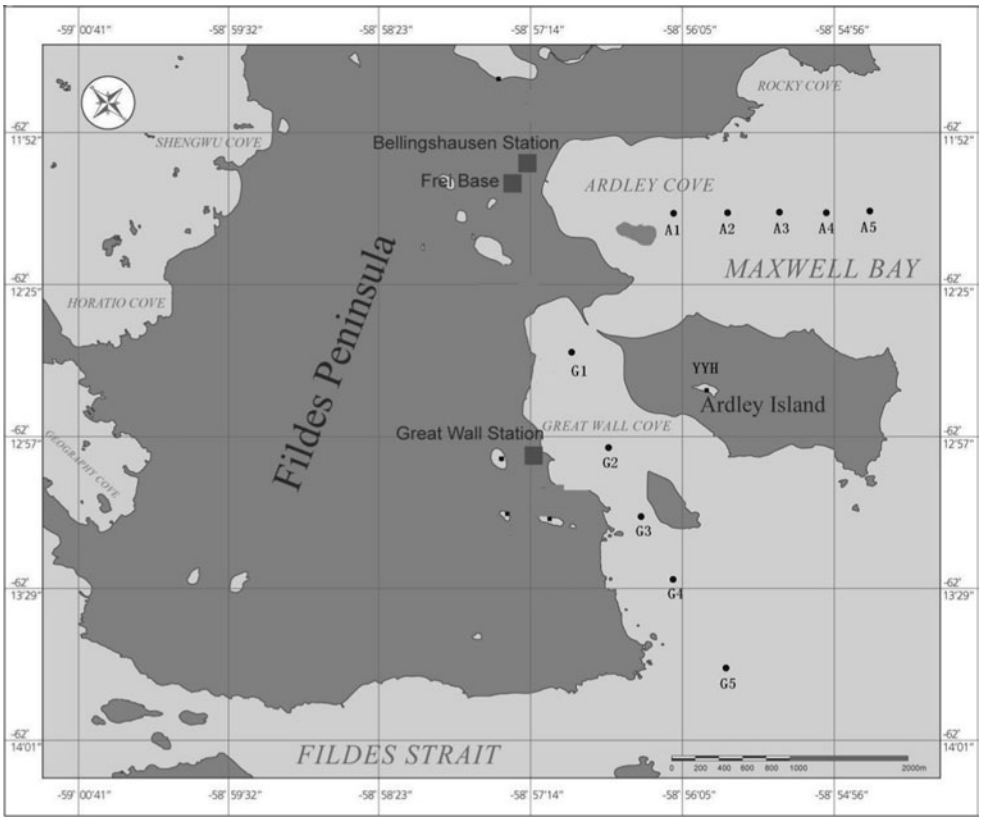

Fig. 1 Location of sampling stations in Ardley Cove and Great Wall Cove, Fildes Peninsula, King George Island, Antarctica

Table 1 Summary of seawater samples collected from Fildes Peninsula (Antarctica) for community structure

Sample	Sampling area	Date	Temperature (°C)	Salinity (psu)	Chlorophyll ($\mu g\ l^{-1}$)	DO ($mg\ l^{-1}$)	SiO_3-Si ($\mu mol\ l^{-1}$)	PO_4-P ($\mu mol\ l^{-1}$)	NO_2-N ($\mu mol\ l^{-1}$)	NO_3-N ($\mu mol\ l^{-1}$)	POC ($\mu g\ l^{-1}$)	Prokaryotes (10^8 cells l^{-1})
G1	Great Wall Cove, Antarctica	2011-12-18	2.0	33.95	0.51	10.41	49.5	1.83	0.18	14.9	59.2	2.953
G2	Great Wall Cove, Antarctica	2011-12-18	2.5	33.96	0.55	9.22	49.5	1.95	0.17	12.1	54.4	2.355
G3	Great Wall Cove, Antarctica	2011-12-18	2.5	34.07	0.51	9.78	30.0	1.85	0.20	11.2	68	2.822
G4	Great Wall Cove, Antarctica	2011-12-18	2.4	34.06	0.74	9.71	44.0	1.88	0.17	14.0	104	2.493
G5	Great Wall Cove, Antarctica	2011-12-18	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.389
A1	Ardley Cove, Antarctica	2011-12-22	1.9	33.95	0.32	9.93	48.7	1.84	0.18	8.6	70.4	3.163
A2	Ardley Cove, Antarctica	2011-12-22	1.6	34.02	0.39	10.83	49.9	1.87	0.18	14.9	49.6	3.343
A3	Ardley Cove, Antarctica	2011-12-22	1.6	34.01	0.32	9.43	41.4	1.87	0.16	13.6	15.2	4.157
A4	Ardley Cove, Antarctica	2011-12-22	1.7	34.07	0.16	9.43	31.5	1.87	0.14	9.7	44.0	5.674
A5	Ardley Cove, Antarctica	2011-12-22	1.5	34.08	0.35	9.78	46.8	1.85	0.15	14.2	49.6	3.518

DO dissolved oxygen, *POC* particulate organic carbon, *ND* no data

filters (Whatman GF/F) and then measured on an elemental analyzer Carlo Erba 1110 (Carlo–Erba Instruments, Milan, Italy). The chlorophyll α concentration was estimated fluorometrically from 20 ml samples filtered through Whatman GF/F filters. The filters were ground in 90 % acetone and left in the dark at −20 °C for 24 h. The fluorescence of the extract was measured with a 10-AU Field Fluorometer (Turner Designs, Sunnyvale, CA, USA). Bacterial abundance was determined using the 4′, 6-diamidino-2-phenylindole staining protocol (Porter and Feig 1980).

DNA extraction and amplification of 16S rRNA genes

Microorganisms present in the sample were collected by filtration of 1.5 l of water onto 0.2-μm-pore-sized Nuclepore filters (Whatman). DNA extraction was performed as described by Bosshard et al. (2000) and Bano and Hollibaugh (2000). A region ~420 bp in the 16S rRNA gene covering the V4 to V5 region was selected to construct a community library through tag pyrosequencing. The bar-coded universal primers 515F and 926R containing the A and B sequencing adaptors (454 Life Sciences) were used to amplify this region. The forward primer (A-515F; Caporaso et al. 2011) was 5′-*CCATCTCATCCCTGCGTGTCTCCGACTCAG*NNNNNNNNNNGTGCCAGCMGCCGCGGTAA-3′, where the sequence of the A adaptor is shown in italics and underlined, whereas the Ns represent a ten-base sample-specific bar code sequence. The reverse primer (B-926R; Liu et al. 2007) was 5′-*CCTATCCCCTGTGTGCCTTGGCAGTCTCAG*CCGTCAATTYYTTTRAGTTT-3′, where the sequence of the B adaptor is shown in italics and underlined. Polymerase chain reactions (PCRs) were conducted in triplicate 20-μl reactions with 0.1 μM each of the primers, ~4 ng of template DNA, 1 × PCR buffer, and 2.5 U of Pfu DNA Polymerase (MBI Fermentas, USA). The amplification program consisted of an initial denaturation step at 95 °C for 1 min, followed by 30 cycles of 94 °C for 30 s (denaturation), 58 °C for 45 s (annealing), and 72 °C for 1 min (extension), and a final extension of 72 °C for 10 min. Amplicons from three PCRs were pooled for each sample. PCR products were purified using a DNA gel extraction kit (Axygen, Hangzhou, China). The DNA concentration of each PCR product was determined using a Quant-iT PicoGreen double-stranded DAN assay (Invitrogen, Germany) and was quality controlled on a TBS-380 Mini-Fluorometer (Turner Biosystems, Sunnyvale, CA, USA). Finally, amplicons of all samples were pooled in equimolar concentrations for pyrosequencing.

Pyrosequencing and data analysis

Amplicon pyrosequencing was performed from the A-end using a Roche 454 Genome Sequencer FLX platform at the Chinese National Human Genome Center in Shanghai. Quality screening was completed in Mothur by removing low-quality reads (Schloss et al. 2009). The valid reads complied with the following rules: Each pyrosequencing read containing a primer sequence was ≥200 bp in length, had no ambiguous bases, matched the primer and one of the used barcode sequences, and was present at least an 80 % match to a previously determined 16S rRNA gene sequence. All 454 sequences were submitted to the Sequence Read Archive database at NCBI (accession no. SRP017315).

Analysis was conducted using the microbial ecology community software program Mothur (Schloss et al. 2009). Sequence reads were compared with a reference database of known 16S rRNA genes [obtained from SILVA and Ribosomal Database Project (RDP) databases] and taxonomically assigned according to the RDP classifier (Wang et al. 2007). Sequences were clustered into operational taxonomic units (OTUs) defined by 97 % similarity. Rarefaction analysis and Good's coverage for the ten libraries were determined. Cluster analysis of the community composition was performed using the statistical software package PAST (http://folk.uio.no/ohammer/past/) with a correlation matrix. A redundancy analysis (RDA) was performed to analyze the variation of communities in the two coves and their relationships with environmental variables using Canoco 4.5 (ter Braak and Šmilauer 2002).

Results

General statistics

Sequence reads with an average of 364 bps were generated after trimming of the primer sequences from the beginning and end of the raw data (Table 2). A total of 112,137 valid reads and 9,043 OTUs (at the 97 % level, corresponding to taxonomically valid species) were obtained from the ten surface seawater samples through 454 pyrosequencing analysis, of which 25,740 reads and 770 OTUs were chloroplasts of eukaryotic algae. In addition, one sequence read belonging to Archaea was detected in the seawater samples.

Higher bacterial richness (Chao value) and diversity (Shannon index) were found in Ardley Cove samples than in Great Wall Cove samples. Good's coverage estimations revealed that 83.30–93.33 % of the species (at the 97 % level) were obtained in all samples. However, rarefaction curves suggest that the sequencing effort was not sufficiently large to capture the complete diversity of these communities because the curves do not level off (the slope does not go to zero) with increasing sample size (data not shown).

Cluster analysis of the bacterial composition at the phylum level revealed a conservation of the community

Table 2 Summary of sequence reads, coverage, and mean values of richness and diversity at the 97 % OTU level of 16S rRNA gene fragments for the ten bacterioplankton communities

Sample	Read number	OTU number	Average length (bp)	Coverage (%)	Richness estimator		Diversity estimator	
					ACE	*Chao1*	*Shannon*	*Simpson*
A1	12,272	2,463	365	87.38	9,726	5,923	6.17	0.008
A2	11,290	1,421	364	93.10	4,158	2,906	5.58	0.010
A3	13,206	2,743	361	88.27	7,927	5,386	6.51	0.005
A4	11,060	2,030	365	89.05	6,717	4,374	6.06	0.006
A5	12,029	1,815	366	92.01	4,799	3,613	5.99	0.007
G1	11,557	1,373	364	93.33	4,102	2,923	5.31	0.015
G2	10,701	1,935	364	89.58	5,851	3,836	5.98	0.008
G3	5,289	1,445	366	83.30	4,733	3,183	6.15	0.006
G4	11,159	2,043	366	88.89	7,379	4,525	5.99	0.008
G5	13,574	1,574	363	93.31	5,174	3,441	5.08	0.033

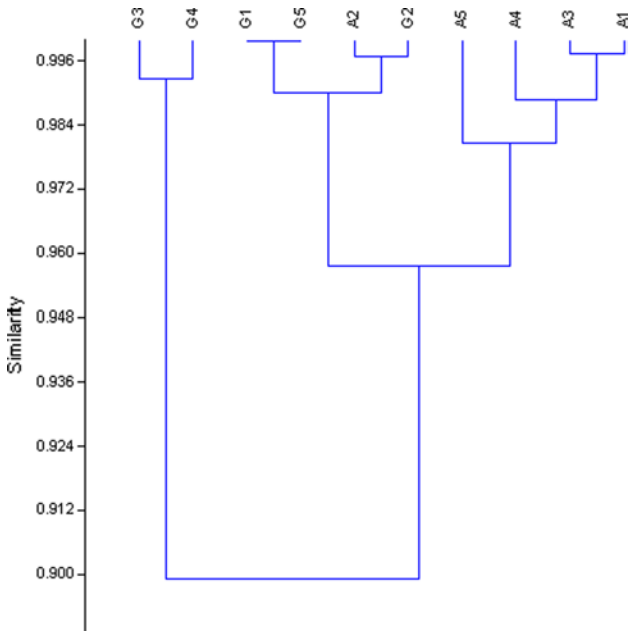

Fig. 2 Cluster analysis of bacterial diversity at the phylum level in the ten sampling sites

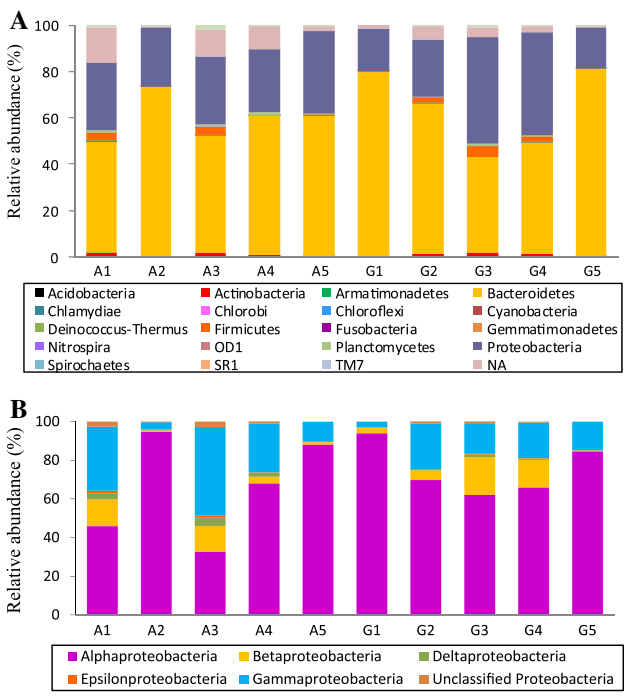

Fig. 3 Comparison of bacterial diversity between the different sampling sites. **a** *Stacked column graph* representing the relative distribution of the bacterial phyla in the different stations. **b** *Stacked column graph* representing the proteobacterial diversity

composition between the two coves (Fig. 2). The stations G3 and G4 in Great Wall Cove were more similar and clustered separately from other stations.

Taxonomic composition

Accounting for 6.3–44.3 % of the total sequence reads from different samples, significant numbers of sequences related to algal chloroplasts were detected in the two Antarctic coves. Chloroplast-related sequences fell into five groups, Bacillariophyta, Chlorophyta, Cryptophyta, Streptophyta, and unclassified chloroplast. Among the algal chloroplasts, Bacillariophyta dominated all stations.

The obtained taxonomy data covered a broad spectrum of known bacterial phyla. Each of the candidate phyla OD1, SR1, and TM7 was presented by only a few sequences (<5 reads). The dominant phyla in all samples belonged to Bacteroidetes (60.7 % of the bacteria on average) and Proteobacteria (29.8 % of the bacteria on average) (Fig. 3a). In addition to the dominant phyla, large numbers of sequence reads related to Actinobacteria, Firmicutes, and Planctomycetes were found in all ten samples.

Sequence reads belonging to Acidobacteria, Armatimonadetes, Chlamydiae, Chlorobi, Chloroflexi, Cyanobacteria, Deinococcus–Thermus, Fusobacteria, Gemmatimonadetes, Nitrospira, Spirochaetes, and Verrucomicrobia were only detected in parts of the ten seawater samples. A large fraction (on average 5.3 % of the bacteria per station) of the bacterial sequence reads could not be assigned below the domain level and were designated as Bacteria NA (not assigned). More abundant reads affiliated with Bacteria NA were observed in Ardley Cove samples than in Great Wall Cove samples.

A total of eight to 18 phyla (12 on average) were determined from the bacterial communities in Ardley Cove, whereas five to 11 phyla (9 on average) were found in the samples from Great Wall Cove. A similar result was observed in proteobacterial diversity in the two coves (Fig. 3B). That is, proteobacterial sequences in all Ardley Cove samples and three Great Wall Cove samples (G3, G4, and G5) fell into the alpha, beta, gamma, delta, and epsilon subclasses. However, neither Delta nor Epsilonproteobacteria were detected in the G1 sample. Epsilonproteobacteria were not observed in the G2 sample.

Table 3 lists the top ten dominant bacterial groups per station, revealing a close relationship between Ardley Cove and Great Wall Cove. Bacteroidetes-related sequences fell into Bacteroidetes_ incertae_sedis, Bacteroidales, Flavobacteriales, Sphingobacteriales, and NA Bacteroidetes. Among the members of Flavobacteriales, Flavobacteriaceae was mainly represented by *Chryseobacterium*, *Flavobacterium*, *Krokinobacter*, *Polaribacter*, *Winogradskyella*, and NA genus, dominating all investigated samples. Cryomorphaceae, which consisted mainly of *Wandonia* and NA genus, was the second most abundant group in Flavobacteriales. Sequences related to Flavobacteriaceae were more abundant in Great Wall Cove (on average 52.2 % of the bacterial reads) than in Ardley Cove (on average 42.6 % of the bacterial reads). By contrast, Cryomorphaceae was more abundant in Ardley Cove than in Great Wall Cove. Among the members of Sphingobacteriales, Saprospiraceae was present at low abundance across all investigated samples. Bacteroidetes_ incertae_sedis and Bacteroidales were represented by only a few sequences (<25) per station.

Among the members of Proteobacteria, Alphaproteobacteria dominated Great Wall Cove (62.1–93.9 % of Proteobacteria) and three stations in Ardley Cove (A2, A4, and A5; 68.0–94.6 % of Proteobacteria). Alphaproteobacteria consisted mainly of organisms belonging to the orders of Caulobacterales, Rhodobacterales, and Sphingomonadales. Represented by *Loktanella*, *Sulfitobacter*, and NA genus, Rhodobacteraceae was abundantly present in all stations (Table 3). In stations A1 and A3 of Ardley Cove, Alpha-, Beta-, and Gammaproteobacteria were more evenly distributed (Fig. 3b). Betaproteobacteria was mainly represented by Burkholderiales (mainly *Polaromonas* and NA genus) and Methylophilales, whereas Gammaproteobacteria consisted mainly of Gammaproteobacteria_incertae_sedis (mainly *Cocleimonas*) and potential novel species, which could not be assigned below the class level. Among the members of Deltaproteobacteria, Desulfobacterales dominated Ardley Cove but was absent in Great Wall Cove. Only 71 reads were affiliated with Epsilonproteobacteria, whereas 225 reads could not be assigned below the Proteobacteria phylum level.

Among the members of Actinobacteria, Actinomycetales was abundantly present at all stations. More sequence reads affiliated to Acidimicrobiales and NA actinobacterial order were detected in Ardley Cove than in Great Wall Cove. Firmicutes was dominated by Bacillales and Clostridiales. *Staphylococcus* and *Clostridium* sensu stricto, accounting for 4.8 and 37.9 % of Firmicutes, respectively, were observed at all stations. Planctomycetes was represented by Planctomycetaceae. More sequence reads assigned to Planctomycetaceae were detected in Ardley Cove than in Great Wall Cove. At the genus level, Planctomycetaceae was mainly represented by the NA genus (86.6 % of Planctomycetes).

Relationship between bacterial community and water properties

The data matrixes of community composition and water properties were tested under a detrended correspondence analysis (DCA) model using Canoco 4.5 to reveal their relationship. Results showed that the length of the first gradient was <3 SD; hence, a linear model was constructed. Hypothesis testing was performed using RDA with Monte Carlo test. For the absence of property data of water, sample G5 was excluded in this analysis. In the RDA ordination (Fig. 4), the eigenvalues for axes 1 and 2 were 0.851 and 0.084, respectively. The species–environment correlations for the first two axes were both 1.00. The first two axes explained 93.6 % of the variance in the species (OTU) data and the same percentage of variance in the species–environment relationship. RDA results demonstrated that DO ($p < 0.05$) accounted for the greatest amount of variability in the coastal bacterial community, followed by NO_3^- and SiO_3^{2-}.

Discussion

Average values of DO and nutrients in Great Wall Cove were similar to those in Ardley Cove, in accordance with the seawater exchange between the two coves when high tide occurs. However, compared with Ardley Cove samples, Great Wall Cove samples showed lower values of bacterial

Table 3 Abundant bacterial groups per station. Groups are given at genus level or at higher taxonomic level if the sequences could not be assigned to a genus

A1		A2		A3		A4		A5	
NA[a]	14.9 (%)	*Polaribacter*	44.2 (%)	Unclassified Flavobacteriaceae	14.5 (%)	*Polaribacter*	24.0 (%)	*Polaribacter*	28.9 (%)
Polaribacter	13.9	Unclassified Flavobacteriaceae	14.1	NA	11.5	Unclassified Flavobacteriaceae	17.6	Unclassified Rhodobacteraceae	19.0
Unclassified Flavobacteriaceae	13.6	Unclassified Rhodobacteraceae	14.1	*Polaribacter*	9.4	Unclassified Rhodobacteraceae	12.9	Unclassified Flavobacteriaceae	15.9
Unclassified Rhodobacteraceae	8.0	*Sulfitobacter*	6.1	Unclassified Cryomorphaceae	8.1	NA	10.1	*Sulfitobacter*	7.0
Unclassified Gammaproteobacteria	6.9	*Flavobacterium*	3.7	Unclassified Gammaproteobacteria	6.7	Unclassified Bacteroidetes	4.1	*Winogradskyella*	3.1
Unclassified Bacteroidetes	5.9	*Winogradskyella*	3.3	Unclassified Rhodobacteraceae	4.9	Unclassified Cryomorphaceae	3.9	*Krokinobacter*	3.0
Unclassified Cryomorphaceae	5.4	*Loktanella*	3.0	*Cocleimonas*	4.5	Unclassified Gammaproteobacteria	3.7	NA	2.2
Sulfitobacter	2.2	*Krokinobacter*	2.0	Unclassified Bacteroidetes	4.1	*Sulfitobacter*	3.7	*Chryseobacterium*	1.8
Clostridium sensu stricto	1.8	Unclassified Cryomorphaceae	1.0	*Wandonia*	2.1	*Wandonia*	2.2	*Cocleimonas*	1.5
Polaromonas	1.6	*Wandonia*	0.7	*Clostridium* sensu stricto	1.6	*Cocleimonas*	1.6	*Loktanella*	1.5
G1		G2		G3		G4		G5	
Polaribacter	63.3 (%)	*Polaribacter*	33.0 (%)	Unclassified Rhodobacteraceae	17.4 (%)	*Polaribacter*	23.2 (%)	*Polaribacter*	61.2 (%)
Unclassified Rhodobacteraceae	9.7	Unclassified Flavobacteriaceae	16.1	*Polaribacter*	16.7	Unclassified Rhodobacteraceae	18.1	Unclassified Flavobacteriaceae	9.5
Unclassified Flavobacteriaceae	7.6	Unclassified Rhodobacteraceae	11.8	Unclassified Flavobacteriaceae	7.1	Unclassified Flavobacteriaceae	11.2	Unclassified Rhodobacteraceae	8.5
Sulfitobacter	6.3	NA	5.6	*Sulfitobacter*	6.5	*Sulfitobacter*	6.2	*Sulfitobacter*	3.7
Krokinobacter	2.5	Unclassified Bacteroidetes	4.1	NA	3.8	*Loktanella*	3.1	*Krokinobacter*	2.1
NA	1.5	*Sulfitobacter*	3.2	*Polaromonas*	3.1	NA	2.5	*Persicivirga*	1.5
Chryseobacterium	1.1	Unclassified Gammaproteobacteria	2.3	Unclassified Gammaproteobacter	2.9	*Alcanivorax*	2.2	*Maribacter*	1.1
Flavobacterium	0.9	*Wandonia*	1.8	Unclassified Cryomorphaceae	2.7	*Polaromonas*	2.1	*Cocleimonas*	1.1
Persicivirga	0.8	Unclassified Cryomorphaceae	1.4	*Flavobacterium*	2.4	Unclassified Bacteroidetes	2.1	NA	0.7
Unclassified Bacteroidetes	0.7	*Cocleimonas*	1.2	*Cocleimonas*	2.3	*Wandonia*	2.0	Unclassified Saprospiraceae	0.6

[a] NA, bacteria not assigned below the domain level

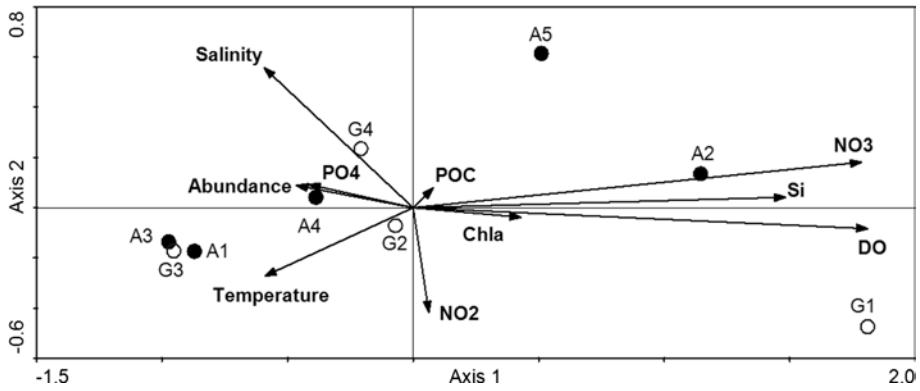

Fig. 4 RDA of the Antarctic bacterial communities as affected by water properties, based on the OTUs (at the 97 % level). Chla, DO, NO_2, NO_3, Si, PO_4, and POC represent chlorophyll, dissolved oxygen, nitrite (NO_2^--N), nitrate (NO_3^--N), silicate (SiO_3^{2-}Si), phosphate (PO_4^{3-}-P), and particulate organic carbon, respectively

abundance and richness but higher concentrations of chlorophyll and POC (Tables 1, 2). This result suggests higher phytoplankton abundance in Great Wall Cove than in Ardley Cove. Bacterial growth potentials are lower under high chlorophyll concentrations, and chemicals produced by phytoplankton can inhibit the growth of competing bacteria (Tada et al. 2011). Bacillariophyta accounted for 97–100 % of the total chloroplast-related sequences in seawater samples. This result is in accordance with a previous study showing that Bacillariophyta members (mainly *Chaetoceros*, *Frigilaria*, and *Thalassiosira*) are dominant in phytoplankton in Great Wall Cove during austral summer (Yu et al. 1999). Phytoplankton blooms dominated by diatoms typically occur in coastal seas (Pinhassi et al. 2004). Phytoplankton biomass accumulates in Great Wall Cove from the mid-December, and significant blooms develop in January (Ma et al. 2013). Cyanobacteria are another primary producer. However, only a few sequence reads could be affiliated with Cyanobacteria in this study, indicating that the Cyanobacteria have been outcompeted by diatoms that were present in large numbers in both Great Wall Cove and Ardley Cove.

Phytoplankton biomass strongly correlated with bulk bacterioplankton growth, and abundance is important in determining the success of different groups and species of bacteria (Pinhassi et al. 2003, 2004). A clear influence of phytoplankton bloom events on bacterioplankton community structure and diversity has been reported in sub-Antarctic Kerguelen Islands and Antarctic Peninsula coastal sites (Ghiglione and Murray 2012). Bacteroidetes members are successful in the degradation of polymeric substances in the ocean (Cottrell and Kirchman 2000b; Pinhassi et al. 2004; González et al. 2008). By contrast, both Alpha- and Gammaproteobacteria seem better adapted to use monomers rather than polymers (Cottrell and Kirchman 2000b). Distinct populations of Bacteroidetes, Alphaproteobacteria, and Gammaproteobacteria are specialized for successive decomposition of algal-derived organic matter (Teeling et al. 2012). In this study, Bacteroidetes, Alphaproteobacteria, and Gammaproteobacteria accounted for 63.6, 20.3, and 5.2 % of the bacterial reads, respectively. Compared with Ardley Cove, higher proportions of Bacteroidetes and Alphaproteobacteria were observed in total bacterial sequences in Great Wall Cove, consistent with the higher concentrations of chlorophyll and POC.

Among the members of Bacteroidetes, *Polaribacter* (55.4 % of Bacteroidetes) dominated all stations (Table 3). A substantial number of genes for adhesion and degradation of polymers, as well as light utilization and sensing, have been detected in *Polaribacter* sp. strain MED 152 (González et al. 2008), suggesting its adaptation in nutrient-rich and nutrient-poor sunlit ocean surface. In addition, an increase in CO_2 fixation in the light is also observed in the *Polaribacter* bacterium (González et al. 2008), indicating a dual life strategy for proteorhodopsin-containing bacteria surviving in oceanic environment. Represented by OTU8667, OTU8870, OTU8910, OTU8997, OTU9009, and OTU9018, 8,509 reads (27.9 % of *Polaribacter*-related clones) showed significant sequence similarity (99.4–100 %) to an uncultured bacterium clone B_NY_1A07 (JN833098) from seawater in the Norwegian Sea (GenBank description), suggesting a bipolar distribution of some *Polaribacter* phylotypes. Another dominant group in Flavobacteriaceae (20.2 % of Bacteroidetes) could not be assigned below the family level (Table 3), suggesting that abundant novel species are present in the investigated area. A similar result was observed in Rhodobacteraceae (accounting for 91.0 % of Alphaproteobacteria), in which the most dominant group (66.6 % of Rhodobacteraceae-related sequences) could not be assigned below the family level (Table 3). *Sulfitobacte*r and *Loktanella* accounted for 25.8 and 5.8 % of Rhodobacteraceae-related sequences, respectively. *Sulfitobacter* and *Loktanella* species are often found in surface waters, and numerous interactions with phytoplankton have been reported (Moran et al. 2007; Boeuf et al. 2013). Known as aerobic anoxygenic phototrophic bacteria, members of the two genera contain low amounts of BChl *a* (Biebl and Wagner-Döbler 2006). In addition, *Sulfitobacter* species generate metabolic energy by sulfite oxidation (Park et al. 2007) and can grow on

dimethylsulfoniopropionate (DMSP) as a sole carbon source. *Loktanella* species can also produce dimethyl sulfide (DMS) when grown on DMSP (Curson et al. 2008). These metabolically versatile bacteria can satisfy a significant part of their carbon and sulfur demands by assimilating DMSP released during the decay of phytoplankton blooms (González et al. 1999; Mou et al. 2005). Members of the genus *Polaribacter* within Flavobacteriaceae and *Sulfitobacter* within Rhodobacteraceae are frequently observed in coastal Antarctic and Arctic marine bacterioplankton communities (Ghiglione et al. 2012; Grzymski et al. 2012; Zeng et al. 2013), suggesting a bipolar distribution of specific species in marine environment.

Among the members of Gammaproteobacteria, NA Gammaproteobacteria accounted for 40.6 % of Gammaproteobacteria-related sequences. Other dominant taxa within Gammaproteobacteria included genera *Cocleimonas* (24.9 %), *Arenicella* (6.4 %), and *Granulosicoccus* (6.2 %). *Cocleimonas*, *Arenicella*, and *Granulosicoccus* species require Na^+ ions for growth (Kurilenko et al. 2010; Romanenko et al. 2010; Tanaka et al. 2011), thus indicating their marine origin. In addition, *Cocleimonas* species are sulfur-oxidizing bacteria (Tanaka et al. 2011), suggesting their relation to sulfur cycling in the Antarctic coastal waters in austral summer.

Represented by *Cocleimonas*-related sequences, Gammaproteobacteria had a higher proportion in bacterial reads of Ardley Cove than in Great Wall Cove. By contrast, a lower proportion of Betaproteobacteria, represented by *Polaromonas*, was observed in Ardley Cove than in Great Wall Cove. The results indicate that bacterioplankton in Great Wall Cove is more influenced by freshwater input than those in Ardley Cove during austral summer. Betaproteobacteria members usually represent a consistently large fraction of bacterioplankton in freshwater lakes and diverse river types (Glöckner et al. 2000; Zwart et al. 2002). They are usually low in abundance in the open ocean. In addition, a few Betaproteobacteria members that have been retrieved from the marine environment are from coastal environments (Rappé et al. 2000; Riemann et al. 2008; Boeuf et al. 2013). *Polaromonas* phylotypes are globally distributed as dormant cells through high-elevation air currents and are commonly found in air and snow samples from high altitudes (Darcy et al. 2011). In this study, represented by OTU7123, a total of 462 reads (82.9 % of the *Polaromonas*-related sequences) showed 100 % identical to *Polaromonas* sp. DAB_10Ecl (JF728953) from glacial surface ice on the same island (GenBank description).

In addition to Cyanobacteria, other diazotrophic bacteria were present in the two investigated coves, of which Rhizobiales and Burkholderiales are known to be associated with plant roots (Franche et al. 2009; Bolhuis and Stal 2011). Whether those bacteria are of terrestrial origin attributing to freshwater input remains uncertain. All collected samples contained considerable numbers of sequence reads related to *Clostridium*, which contains some nitrogen-fixing species (Chen et al. 2001). Whether anaerobic ammonium oxidation occurs in the investigated area remains uncertain because no sequence read was found directly related to the known anammox species among the Planctomycetes. The largest fraction of Planctomycetes (~377 reads) could not be assigned below the family level.

In summary, a high diversity of bacterioplankton in Great Wall Cove and Ardley Cove of the Antarctic King George Island was detected for the first time by using high-throughput pyrosequencing. More than 70 % of the bacterial community consisted of chemoheterotrophs (mainly Flavobacteriales) and photoheterotrophs (mainly Rhodobacterales). These results are in agreement with previous study showing that inferred metabolisms of summer bacterioplankton in Antarctica Peninsula coastal surface waters were characterized by chemoheterotrophy, photoheterotrophy, and aerobic anoxygenic photosynthesis (Grzymski et al. 2012). Chloroplast-related sequences accounted for more than 20 % of the total sequence reads, indicating the occurrence of phytoplankton bloom at the time of sampling. Similar nutrient concentrations were present in the two coves, and they also had similar bacterioplankton community structures. However, compared with Ardley Cove, Great Wall Cove with higher chlorophyll and POC concentrations exhibited relatively low bacterial richness and diversity. Some cosmopolitan species including *Polaribacter* and *Sulfitobacter* may have similar ecological functions in similar marine environments.

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