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# Distinct Patterns of Marine Bacterial Communities in the South and North Pacific Oceans[§]

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**The study of oceanic microbial communities is crucial for our understanding of the role of microbes in terms of biomass, diversity and ecosystem function. In this study, 16S rRNA gene tag pyrosequencing was used to investigate change in bacterial community structure between summer and winter water masses from Gosung Bay in the South Sea of Korea and Chuuk in Micronesia, located in the North and South Pacific Oceans, respectively. Summer and winter sampling from each water mass revealed highly diverse bacterial communities, containing ~900 Operational Taxonomic Units (OTUs). The microbial distribution and highly heterogeneous composition observed at both sampling sites were different from those of most macroorganisms. The bacterial communities in the seawater at both sites were most abundant in *Proteobacteria* during the summer in Gosung and in *Bacterioidetes* during the winter. The proportion of *Cyanobacteria* was higher in summer than in winter in Chuuk and similar in Gosung. Additionally, the microbial community during summer in Gosung was significantly different from other communities observed based on the unweighted UniFrac distance. These data suggest that in both oceanic areas sampled, the bacterial communities had distinct distribution patterns with spatially- and temporally-heterogeneous distributions.**

***Keywords***: Operational Taxonomic Units (OTUs), pyrosequencing, 16S rRNA

## Introduction

Survey of diversity patterns and ranges of marine microbes in seawater masses is crucial for understanding the evolutionary and ecological processes that shape contemporary biodiversity, and for monitoring the response of marine ecosystems to future environmental changes. Recent advances in next-generation sequencing techniques have allowed large-ale exploration of taxonomic diversity and geographic distribution of marine microbes (Sunagawa *et al*., 2010; Chen *et al*., 2011; Emami *et al*., 2012). High-throughput pyrosequencing techniques for phylogenetically informative marker genes, such as the ribosomal RNA gene (rRNA), have been employed to characterize the genetic diversity, community composition, relative abundance, and distribution of microbes in the ocean water masses (Li *et al*., 2006; Lee *et al*., 2012; McKew *et al*., 2012; Morrow *et al*., 2012).

The ocean is the largest environment on Earth, the water masses of which are characterized by physical properties such as strong physical mixing due to currents and storms, variable nutrient states, and the occurrence of widely distributed microbes. The Baas-Becking and Beijerink tenet "everything is everywhere, but, the environment selects" (Becking, 1934; Finlay, 2002) may explain the apparent cosmopolitan biogeography of microorganisms by invoking a lack of dispersal limitation and imposition of regional distinctions by environmental selection. However, recent arguments for this principal have cited molecular evidence of microbial biogeographical patterns that suggest limited gene flow and limited potential for dispersal of universal marine organisms such as diatom (Martiny *et al*., 2006; Amaral-Zettler *et al*., 2010; Casteleyn *et al*., 2010). Furthermore, the recent use of deep sequencing to determine microbial community structures in the ocean demonstrated limited microbial distribution and a highly heterogeneous composition (Sul *et al*., 2013). The barriers to dispersal of marine microbes may be the water masses themselves, such as the ocean currents that occur at the equator, and in some cases the geochemical barriers that limit the distribution of certain circulation patterns. However, although the biogeography of marine bacteria exhibits a limited pattern in terms of latitude, some abundant bacteria tend to be distributed more widely across datasets and have latitudinal range sizes (Finlay, 2002; Pommier *et al*., 2007; Ladau *et al*., 2013; Sul *et al*., 2013). This indicates that abundant bacteria may migrate to, or emigrate from, adjacent regions by means of strong passive transport mechanisms such as thermohaline circulation and oceanic currents. Other driving forces of marine bacterial biogeography likely include environmental differences in temperature and water mass composition. For example, a global warming climate (affecting ocean temperature, salinity, pH levels, and current circulation patterns) can significantly change the patterns of microbial distribution

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and disturb the marine ecosystem.

This study investigated the diversity and structure of bacterial communities associated with seasonal marine surface waters from the North and South Pacific Oceans (represented by Gosung Bay in the South Sea of Korea and Chuuk in Micronesia, respectively), the microbial consortia of which have been documented rarely. The data showed that in both Pacific ocean areas, bacterial communities have distinct distribution patterns and highly heterogeneous compositions. These findings contribute to knowledge of marine microbe biogeographical patterns that may be tested in more comprehensive studies.

## Materials and Methods

### Sample collection

Surface seawater (0–5 m) was collected in the winter (11th January) and summer (21st August) of 2013 from the South Sea areas of Gosung, Korea (N34°55′38.7″E128°18′54.9″, N34°52″02.2″E128°14′55.1″) and in winter (22nd January) and summer (9th August) from Chuuk Island in Micronesia (N7°15′13.1″E151°83′60.4″, N7°46′11.7″E151°86′12.8″). All samples were prepared from each site to identify significant effects and a higher frequency of false negatives. Ambient seawater (~30 L) was collected into sterile plastic bottles and filtered through a polycarbonate filter membrane (0.22 μm; Millipore, USA). All samples were stored at -80°C.

### DNA extraction, PCR amplification, and pyrosequencing

The filter membranes with the adsorbed microbial cells were cut into pieces prior to DNA extraction. Total DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio, USA) according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification was performed using the extracted total DNA with primers targeting the V1-V3 regions of the 16S rRNA gene. For the bacterial amplification, the barcoded primers 9F (5′-CCTATCCCCTG TGTGCCTTGGCAGTC-TCAG-AC-AGAGTTTGATCM TGGCTCAG-3′; the underlined sequence indicates the target region primer; 'CCTATCCCCTGTGTGCCTTGGCAG TC' indicates the adaptor sequence; 'TCAG' is the key sequence; 'AC' is the linker sequence) and 541R (5′-CCATC TCATCCCTGCGTGTCCGAC-TCAG-X-AC-ATTACCG CGGCTGCTGG-3′; 'X' indicates the unique barcode for each subject; 'TCAG' indicates the key sequence; 'CCATC TCATCCCTGCGTGTCCGAC' is the dose adaptor sequence; 'AC' is the dose linker sequence; 'ATTACCGCGGCTGCT GG' is the dose primer sequence) were used. Amplification was performed under the following conditions: initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 30 sec, primer annealing at 55°C for 30 sec, and extension at 72°C for 30 sec, with a final elongation at 72°C for 5 min. The PCR products were confirmed by electrophoresis on 2% agarose gels and visualized using the Gel Doc system (Bio-Rad, USA). The PCR products were extracted from the agarose gels using the QIAquick PCR Purification Kit (QIAGEN, Cat. # 28106). Equal amounts of purified products were pooled, and short fragments (non-target products) were removed using the Ampure bead kit (Agencourt Bioscience, USA). The product size and quality were assessed on a Bioanalyser 2100 (Agilent, USA) using a DNA 7500 chip. Mixed amplicons were subjected to emulsion PCR and then deposited on picotiter plates (Agilent). Sequencing was performed by Chunlab Inc. (Korea) using the GS Junior Sequencing system (Roche Branford, USA) according to the manufacturer's instructions.

### Pyrosequencing data analysis

The basic analysis was conducted as described previously (Chen *et al*., 2011; Hur *et al*., 2011; Kim *et al*., 2012a, 2012b). Obtained reads were sorted using the unique barcode of each PCR product. The sequences of the barcode, linker, and primers were removed from the original sequencing reads. Any reads containing two or more ambiguous nucleotides, a low quality score (average score < 25), or reads shorter than 300 bp, were discarded. Potential chimera sequences were detected by the Bellerophon method, which compares the BLASTN search results between the forward-half and reverse-half sequences (Hurber *et al*., 2004). After removal of chimeric sequences, the individual reads were assigned their taxonomic positions according to the highest pairwise similarity among the top BLASTN hits against the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net) (Kim *et al*., 2012a, 2012b). The richness and diversity of samples were determined by Chao1 richness estimation and Shannon diversity index at a distance of 3%. Random subsampling was conducted to equalize the read size of samples for comparison of different read sizes among samples. The overall phylogenetic distance between communities was estimated using the Fast UniFrac (Hamady *et al*., 2010) and visualized using principal coordinate analysis (PCoA). To compare OTUs between samples, shared OTUs were identified by XOR analysis (CL community program; Chunlab Inc.)

**Table 1. Numbers of sequences and OTUs (97%) and diversity estimates of bacteria**

| Index | Chuuk | | | | Gosung | | | |
|---|---|---|---|---|---|---|---|---|
| | Winter | | Summer | | Winter | | Summer | |
| | Site 1 | Site 2 | Site 1 | Site 2 | Site 1 | Site 2 | Site 1 | Site 2 |
| No. of Seq | 4640 | 7410 | 13374 | 17370 | 5156 | 6286 | 4662 | 9266 |
| OTUs | 633 | 1886 | 786 | 902 | 646 | 762 | 652 | 884 |
| Ace | 3111.3 | 8829.2 | 2126.8 | 2000.9 | 1731.5 | 1420.3 | 1808.9 | 1979 |
| Chao1 | 2376.8 | 4766.2 | 1513.8 | 1612.6 | 1087.3 | 1247.9 | 1228.2 | 1486.7 |
| Shannon | 5.332 | 6.008 | 4.119 | 4.608 | 4.648 | 5.154 | 4.726 | 4.800 |

Numbers of sequences and OTUs (97%) and diversity(TDC-TBC) estimates of bacteria

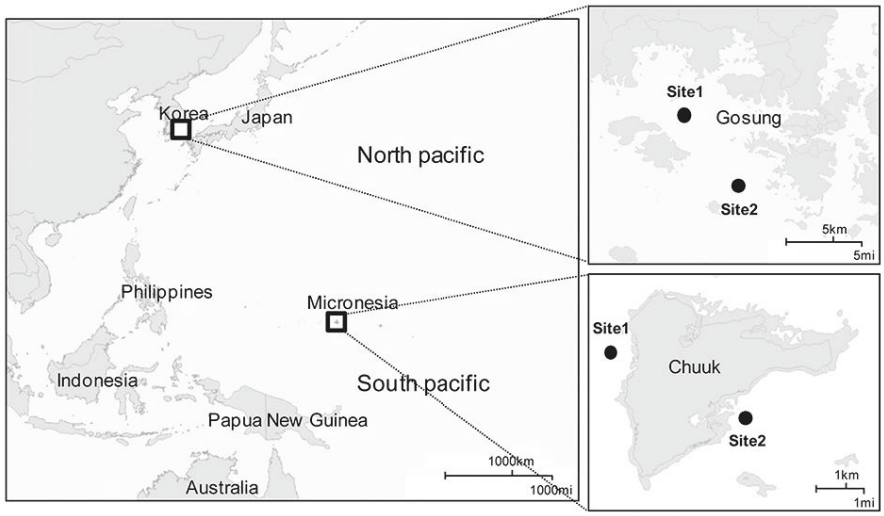


**Fig. 1.** Maps showing the sampling locations and environmental index in North and South Pacific Oceans.

| Location | Season | Temperature (°C) | Salinity (‰) | DO (mg/L) | Chlorophyll a (μg/L) |
|---|---|---|---|---|---|
| Gosung | Winter | 7.62±0.92 | 34.1±0.02 | 9.24±0.15 | 0.67±0.15 |
| | Summer | 26.2±0.21 | 33.6±0.03 | 7.98±0.13 | 1.27±0.11 |
| Chuuk | Winter | 28.6±0.13 | 34.0±0.02 | 6.52±0.06 | 0.31±0.03 |
| | Summer | 29.9±0.08 | 34.1±0.01 | 5.21±0.13 | 0.39±0.06 |

Temperature, salinity, DO, and Chlorophyll a content were measured with a YSI 6600 Sonde. The data presented are means (±standard deviation) of 6 measurements: 3 measurements per each site, site1 and site2.

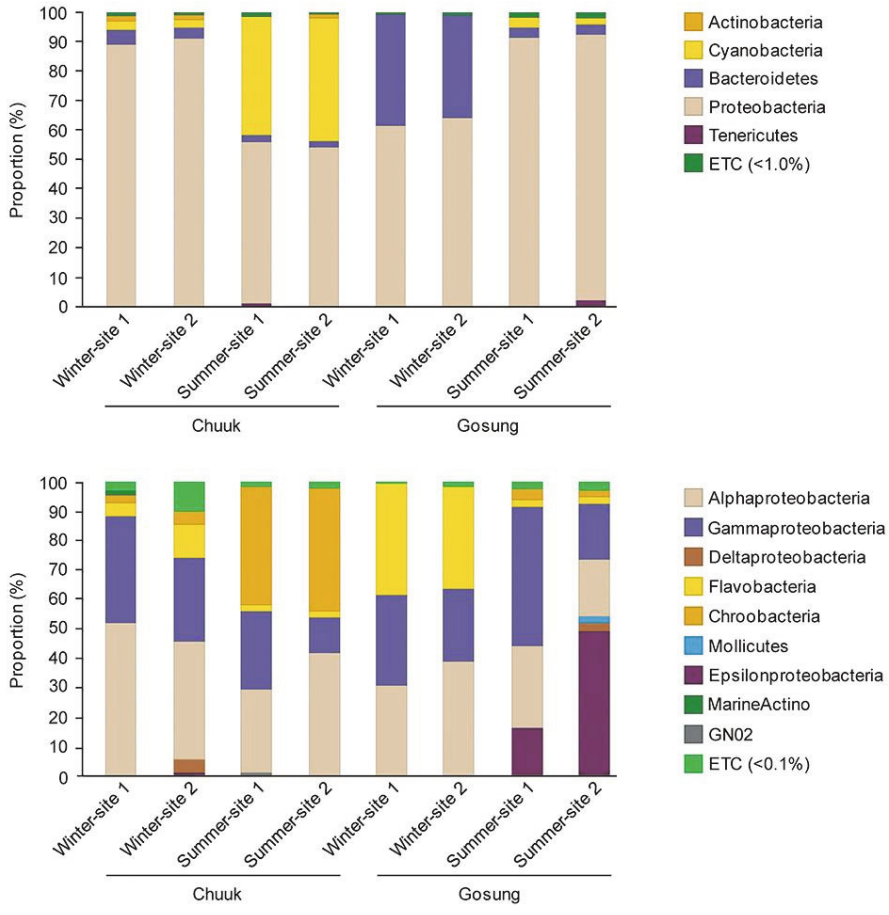


**Fig. 2.** Taxonomic classification of bacterial reads retrieved from pooled DNA amplicons from different seasonal water masses into phylum (A) and class (B) levels using the RDP classifier.

## Results

### Diversity and composition of seawater-associated bacterial communities

To determine the bacterial composition and distribution of water masses, samples were collected in winter and summer at two sites, Gosung and Micronesia in which the environmental factors including temperature, dissolved oxygen (DO) and chlorophyll-a differed significantly (one-way ANOVA; $P < 0.05$) (Fig. 1). In particular, all environmental parameters between winter and summer in Gosung differed significantly, not in Chuuk. Pyrosequencing was performed on the mixed PCR amplicons generated from the pooled DNA. A total of 68164 reads (average number of reads per sample, 8520; average read length, 372 bp) were recovered after quality filtering, and clustered into 7151 OTUs (97% of the qualified reads; Table 1). There were 4207 and 2944 OTUs in samples collected in Micronesia and Gosung, respectively. The numbers of seasonal OTUs in winter and summer were 2519 and 1688 OTUs from Micronesia, and 1408 and 1536 from Gosung, respectively. According to the nonparameteric Chao1 index, bacterial richness of the entire bacterial community was highest in Chuuk during winter compared to other samples. Additionally, similar trends in the Shannon (diversity index) were observed between the sampling sites or between seasons, with the exception of in the winter water masses collected from Micronesia, which showed much more bacterial diversity and even than the others.

A total of 68164 reads were assigned to 13 formally described bacterial phyla, and an average of 97.8% was affiliated with five ubiquitous phyla (*Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Actinobacteria, Tenericutes*) (Fig. 2A). The proportions of these five phyla varied among different seasonal waters from the two sites; however, *Proteobacteria* was most abundant, comprising 54–90% of the qualified bacterial reads in all seawater samples. Interestingly, its proportion was decreased during summer in Chuuk compared with winter, while increased in Gosung. The second most abundant group was *Cyanobacteria*, representing 40.9% of reads in the summer seawater of Chuuk, while its proportion in all other samples was negligible. *Bacteroidets* was more abundant during winter in Gosung than the other samples (Fig. 2A). Further classification at the class level indicated that bacterial communities varied considerably between winter and summer in Chuuk and Gosung (Fig. 2B). For instance, summer seawater from Chuuk was dominated by *Chroobacteria*, with the majority of reads belonging to the phyla *Cyanobacteria*; whereas winter seawater contained varying proportions of *Alphaproteobacteria* and *Gammaproteobacteria* of the phylum *Proteobacteria*. In the Gosung seawater, *Epsilonproteobacteria* of the phylum *Proteobacteria* were most abundant in summer; whereas *Flavobacteria* and *Alphaproteobacteria* were heavily represented in winter. In particular, *Deltaproteobacteria* and *Chroobacteria* were not present during summer in Gosung.

Next, heat map analysis of the bacterial communities in seawater at the two sites showed a distinct bacterial distribution pattern between North and South pacific oceans at the genus level. For example, *Cyanobacteria* was abundant during summer in Chuuk (Fig. 3). With the exception of *Pelagibacter* (which was distributed in all samples), further classification at the genus level revealed distinct seasonal diversities of all bacterial taxa with a spatially and temporally heterogeneous distribution (Fig. 3). For example, more sequences associated with *Prochlorococcus* were detected in Chuuk during summer compared to other water masses; while *Roseovarius*, *Marinobacterium*, *Halomonas*, and *Marivita* were dominant during winter in Chuuk. *Alteromonas* was more abundant in Chuuk than Gosung. In Gosung, *Arcobacter*, *Vibrio*, *Thalassospira*, *Amphritea*, and *Aliivibrio* were relatively abundant during summer; whereas *Jannaschia*, *Glaciecola*, *Polaribacter*, *ABCO*, and *Olleya* were most abundant during winter. The abundance of *Pseudoalteromonas* was higher during both seasons in Gosung compared to Chuuk.

### Distinct distribution pattern of bacterial communities in the South and North Pacific Oceans

We further investigated the relative abundance and diversity patterns of the microbial communities in Chuuk and Gosung, representing the South and North Pacific Oceans,

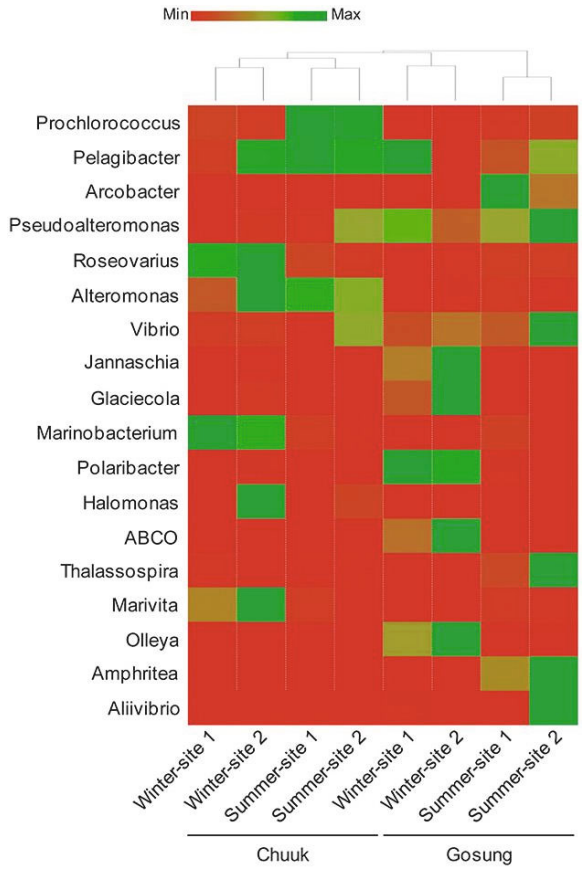


**Fig. 3. Heat map showing the relative abundances and distribution of representative 16S rRNA gene tag sequences classified at the genus level.** A divergence setting of > 5% was used to filter out genera with small differences among the samples. The normalized data were centered by mean and clustered using the complete linkage method and a metric of correlation (uncentered). The color code indicates differences of the relative abundance from the mean, ranging from green (negative) through black (the mean) to red (positive).

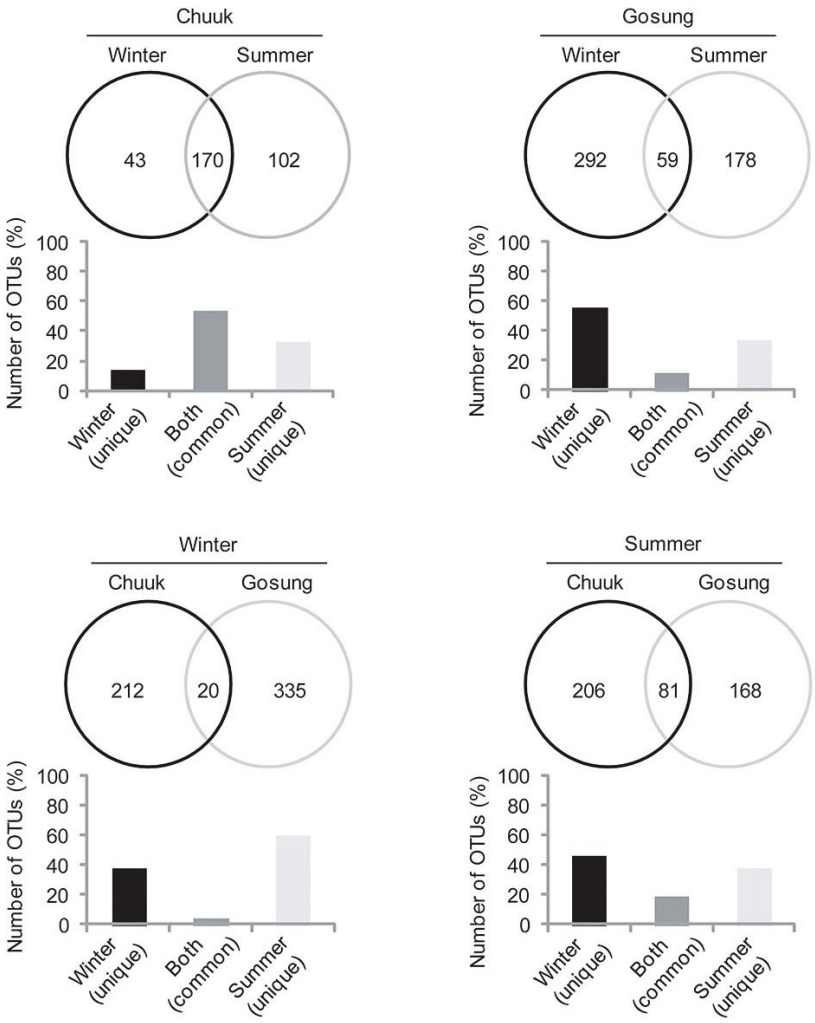


**Fig. 4. The distribution of common and unique bacterial groups in the seasonal bacterial communities (A) and spatial bacterial communities (B) in North or South pacific ocean.**

respectively. The distribution of OTUs within the seawater samples was investigated by combining all tag sequences and determining their presence in different samples. With regard to seasonal differences in bacterial diversity, in Chuuk the number of OTUs which are present in summer and absent in otherwise winter (102) were higher than that of OTUs in winter (43); while the opposite pattern in Gosung was observed (Fig. 4A). Both seasonal water masses shared 171 OTUs in Chuuk, 163 of which were mainly distributed in *Alphaproteobacteria* (91), *Gammaproteobacteria* (40), *Chroobacteria* (15), *Flavobacteria* (17), whereas the other could be identified as minor bacterial groups such as MarineActino and *Chlorophyta*. In Gosung 54 of the shared OTUs (59) were distributed in *Gammaproteobacteria* (44) and *Alphaproteobacteria* (10) (Supplementary data Table S1). While the proportion of OTUs common to both seasons in Gosung were present at less abundances compared to bacteria unique to each season, the opposite distributional pattern in Chuuk was observed, indicating that common OTUs in Gosung were less richness than Chuuk. Additionally, with regard to the spatial distribution of bacterial communities (Fig. 4B), some coexisted in both the South and North Pacific Ocean sites, but most showed a spatially heterogeneous distribution. The number of common OTUs increased dramatically during summer compared to winter (Fig. 4B). Both winter samples shared only 19 OTUs in Chuuk, 17 of which were distributed in *Alphaproteobacteria*, whereas the other were identified as *Alphaprobacteria* and *Flavobacteria* (Supplementary data Table S1). In the summer samples, 80 of the shared OTUs (81) were distributed in *Alphaproteobacteria* (49), *Chroobacteria* (16), *Gammaproteobacteria* (9) and *Flavobacteria* (6).

Next, to compare bacterial communities in seawater samples from different spatial habitats, a UniFrac distance-based jackknife cluster was computed (Fig. 5). Bacterial communities from Chuuk seawater samples generally clustered together, indicating a high degree of similarity. This cluster was well separated from that formed by the samples from Gosung during winter and summer, suggesting substantial dissimilarity between the bacterial communities in Gosung and Chuuk. In particular, the microbial community associated with the Gosung water mass in summer was significantly different from other communities, as indicated by the principal coordinate analysis based on unweighted UniFrac distance.

The principal component analysis (PCA) results of several

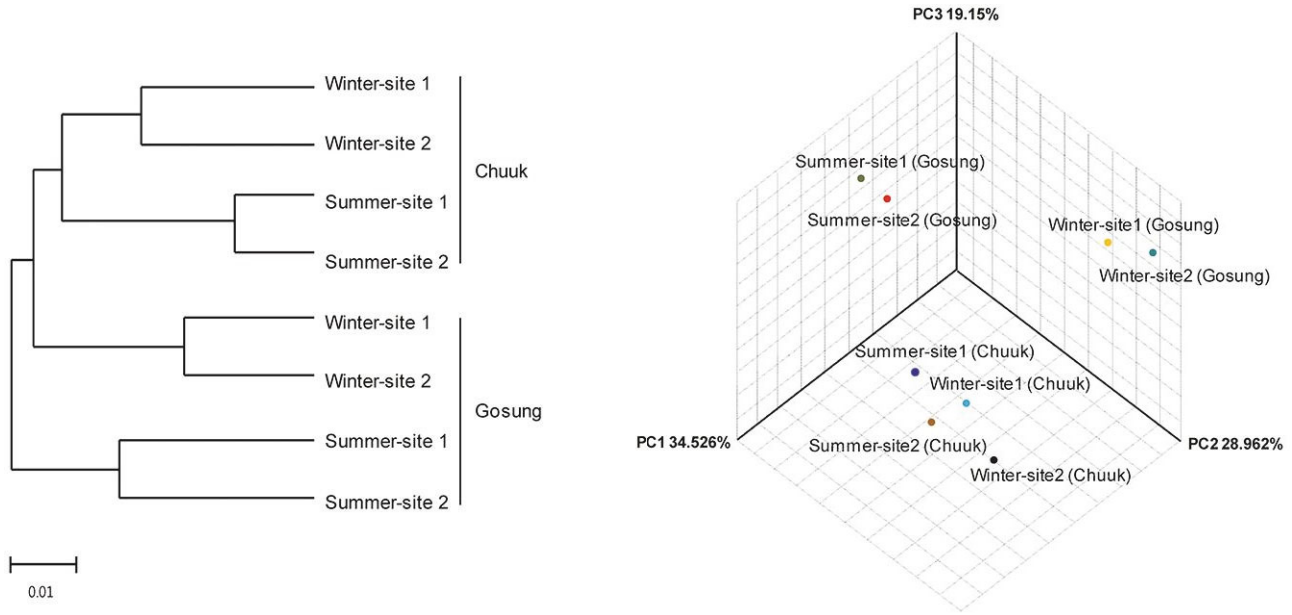


**Fig. 5. UniFrac distance-based Jacknife clustering of bacterial communities associated with different seasonal water masses from different sampling locations.**

main microbial group assemblages in response to different environmental factors are shown in Supplementary data Fig. S1. Correlations between specific environmental factors and microbial groups are represented by the angle of the arrows between them. Chlorophyll a contributed strongly in the spatial distribution of *Peudoalteromonas*, *Vibrio*, *Amphritea*, and *Aliivibrio*, while temperature had a negative contribution to the distribution of *Jannachia*, *Glacieco*, *Marinobacterium*, *Polaribacter*, *Halomonas*, *Marivita*, *Roseovarius*, and *Olleya*. On the other hand, *Alteromonas* and *Prochlorococcus* showed notable negative correlations with chlorophyll a and dissolved oxygen (DO), respectively.

## Discussion

Marine microbes play an important role in energy and matter fluxes in the sea; thus an understanding of their distribution and seasonal patterns of diversity is required. Many studies have investigated microbial distribution and diversity with regard to the distinct environmental and geographical conditions in various microbial habitats (Harvell *et al*., 1999; Harley *et al*., 2006; Danovaro *et al*., 2011; Giovannoni and Vergin, 2012). In this study, bacterial communities associated with seasonal seawaters from the South and North Pacific Oceans were investigated. In previous studies, seasonal changes in bacterial diversity were suggested by shifts in the relative abundance of OTUs (Giovannoni and Vergin, 2012). The global marine bacterial diversity peaks at high latitudes in winter (Ladau *et al*., 2013), differing from the seasonally consistent and high diversity of macroorganisms (Pommier *et al*., 2007; Fuhrman *et al*., 2008). However, this study found that marine bacterial diversity at temperate latitudes was consistent between winter and summer. By contrast, the number of OTUs detected during winter in Micronesia was higher than that in Gosung, as indicated by the higher Shannon and Chao1 values than those of other ocean water masses. Based on the variability among phyla, we generated separate diversity maps for the dominant bacterial phyla to determine whether the patterns of diversity were consistent among phyla. Diverse phylum-specific diversity patterns were found, likely reflecting the marked functional diversity encompassed by bacteria. In accordance with previous studies of the relative richness of dominant phyla (Emami *et al*., 2012; Ladau *et al*., 2013), *Proteobacteria* were the dominant bacterial phylum and qualitatively similar in all water masses, whereas the patterns of other phyla were unique. For example, *Cyanobacteria* richness was highest in the tropical latitudes, particularly during summer, whereas the richness of the *Gammaproteobacteria* and *Bacteroidetes* peaked at high latitudes. In the samples collected from Micronesia during summer, the cyanobacterial genus *Prochlorococcus* had high relative abundance in tropical waters but not elsewhere, in agreement with a previous report (Johnson *et al*., 2006). The peaks of *Prochlorococcus* at tropical latitudes suggest the involvement of light availability in the evolution of distinct *Prochlorococcus* ecotypes (Johnson *et al*., 2006). By contrast, the *Alphaproteobacteria* genus *Pelagibacter* is distributed widely in all seasons (Ladau *et al*., 2013), but exhibits a pronounced peak in relative abundance in Micronesia during summer. The high relative abundance may contribute to the low Shannon diversity in Micronesia during summer compared to winter (Table 1). In agreement with the notion that summertime blooms reduce the Shannon diversity at high latitudes (Barz *et al*., 2010), *Acrobacter* was abundant during summer in the North Pacific Ocean (Gosung), but exhibited low relative abundance during winter. In the present study, some bacteria were present during summer or winter at both sites, although most showed spatially heterogeneous distributions. The number of common

bacteria increased dramatically during summer compared to winter, as indicated by the low bacterial diversity in Micronesia during winter. These patterns differ considerably from those reported previously (Ghiglione and Murray, 2012; Ladau *et al*., 2013) and they do reverse on a seasonal basis. The inconsistency of the findings for the specificity of seasonally associated microbial communities may be due to different environmental parameters at two sites. In this study, water masses were collected at low latitude, whereas in the other studies, samples were collected at high latitudes. Environmental factors may affect the distribution and abundance of marine bacteria, particularly pathogenic bacteria. For example, PCA ordination revealed that chlorophyll a contributed strongly to the spatial distribution of *Peudoalteromonas*, *Vibrio*, *Amphritea*, and *Aliivibrio*, while temperature had a negative effect on the distribution of *Glacieco*, *Marinobacterium*, *Polaribacter*, *Halomonas*, *Marivita*, *Roseovarius*, and *Olleya*. In general, the primary product of the oceans represents an energy source available to bacteria, and chlorophyll a may also interact to some extent with phytoplankton, which has an important influence on bacterial growth and production (Lindström, 2001; Crump *et al*., 2003). A dramatic change in chlorophyll a levels during the summer in Gosung may lead to an increase in the growth of *Peudoalteromonas*, *Vibrio*, *Amphritea*, and *Aliivibrio*. On the other hand, a high number of *Glaciecola* and *Polaribacter* were present in the cold water winter samples, which was consistent with an earlier report (Brinkmeyer *et al*., 2003). In addition, in Gosung the proportion of *Vibrio* spp. increased significantly during the summer compared with the winter. The effect of temperature on the abundance of *Vibrio* spp. in marine water was investigated (Motes *et al*., 1998; Johnson *et al*., 2010). An elevated water temperature resulted in a notable increase in the incidence of yellow blotch disease (YBD) on inoculated corals infected with *Vibrio* spp. The number of *Vibrio* spp. in the ocean strongly correlated with water temperature, until the temperature reached 26°C, above which there appeared to be no additional increase in the number of bacteria (Motes *et al*., 1998; Johnson *et al*., 2010). Since water temperature normally exceeds 26°C from May through to October in the North Pacific Ocean, *Vibrio* density could increase during the summer. In fact, *Vibrio* spp. was predominant in the samples collected during the summer from the North Pacific Ocean, indicating that geographical environmental factors, such as temperature, may be strongly associated with *Vibrio* density (Motes *et al*., 1998). In contrast, in the South Pacific Ocean, the proportion of *Vibrio* spp. remained at a constant, low level when temperatures exceeded 26°C during the winter and summer. The susceptibility of marine organisms to disease could increase due to changes in environmental conditions that either increase the prevalence and virulence of existing pathogenic bacteria or facilitate the introduction of other pathogens, including *Vibrio* and *Acrobacter*. During the summer, the proportion of *Vibrio* and *Acrobacter* were markedly higher in Gosung compared with Micronesia. This study provides novel insight into the seasonal changes involved in the structure of bacterial communities associated with different spatial habitats.

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