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Full Length Research Paper

Spatial variation of bacterial community composition near the Luzon strait assessed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and multivariate analyses

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Different water masses have distinct natural physical and chemical properties, which may influence the bacterial community structure. In the western pacific on both sides of the Luzon strait, which is covered with three water masses including the South China Sea (SCS) water mass, the Kuroshio water mass and the SCS-Kuroshio transition water mass, we selected 11 stations to estimate the spatial variation of surface water bacterial community composition using denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified bacterial 16S ribosomal deoxyribonucleic acid (DNA) gene fragments and interpreted the results; its relationship with physical and chemical factors based on multivariate statistical analysis. A total of 343 bands at 105 different positions were detected in DGGE profiles and 18 distinct DGGE bands were sequenced. Sequence analysis revealed that most of the phylotypes were typical marine uncultured bacteria, and predominant bacteria came from three major phyla; *Proteobacteria*, *Actinobacteria* and *Cyanobacteria*. Apparent phylotype richness near the Luzon strait area varied between 24 and 35 bands (mean 31). Similarity and cluster analysis indicate that the distribution and composition of bacterial community at transition area were more affected by the SCS water mass than Kuroshio current. Redundancy analysis (RDA) revealed that temperature, salinity and the concentration of nitrate accounted for a significant amount of the variability in the bacterial community composition (P=0.004, P=0.014 and P=0.038, respectively, P<0.05).

Key words: Bacterial community composition, spatial distribution, Luzon strait; Kuroshio Current, denaturing gradient gel electrophoresis (DGGE), redundancy analysis (RDA).

INTRODUCTION

The Kuroshio, is characterized by high speed, narrow width and great depth, and is the strongest western boundary current of the North Pacific Ocean (Chen et al., 1995). It brings a large quantity of warm equatorial seawater while traveling northward along the east coast of Taiwan (Pai et al., 1987). In addition to its high temperature, salinity and nutrients, the Kuroshio has important impacts both on the local climate and the marine biological resources (Chen et al., 1994). The Luzon strait connects the Philippine sea in the western Pacific to the South China Sea (SCS) between Taiwan

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and north of the Philippines. The northward Kuroshio can intrude into the SCS through the Luzon strait (Liang et al., 2008), so it forms different water masses with different natural physical and chemical properties on the two sides of Luzon strait. There are SCS water mass, the Kuroshio water mass and the SCS-Kuroshio transition water mass. Bacteria are recognized to be important agents of biogeochemical changes in marine ecosystems. They are the important members of the microbial loop and act as a food source for higher tropic levels (Cole et al., 1988). Studying the marine bacterial diversity will certainly help to better understand the function of marine ecosystem. The activity of the microbial community is influenced by the community composition in relation with abiotic (for example, availability of nutrients) and biotic factors (for example, grazers) (Sinsabaugh and Foreman, 2001). Therefore, composition of bacterial communities can vary significantly among water masses due to their different hydrographic properties (Kataoka et al., 2009). However, little information of spatial variation of bacterial community composition among different water masses on both sides of the Luzon strait is known at present. Only about 1% of bacteria in nature can be cultured (Rusch et al., 2007).

In order to overcome the severe limitations of culture dependant methods, denaturing gradient gel electrophoresis (DGGE), a culture-independent genetic fingerprinting technique, was introduced for investigating the diversity of bacterial assemblages (Muyzer et al., 1993; Muyzer and Smalla, 1998; Murray et al., 1996; Moeseneder et al., 1999; Riemann et al., 1999; Schauer et al., 2000). It has been proved to be a high resolution isolation means and can demonstrated that bacterial populations represent less than 1% of the total community (Muyzer et al., 1993; Murray et al., 1996; Miletto et al., 2007 Taketani et al., 2010). Statistical analysis facilitates the analysis of the relationship between the environmental factors and members of bacterial community (Kataoka et al., 2009).

The combination of the molecular method with multivariate analysis method has been used to investigate different microbial communities in different communities such as sediment nitrogen-fixing bacterial community (Zhang et al., 2008), composting bacterial and fungal community (Zhang et al., 2011), copper mining wastewater (Islam-ud-din et al., 2010) and bacterial community of different water masses (Liu et al., 2008; Kataoka et al., 2009). Using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), sequencing and multivariate statistical analysis, we conducted this study with the aim of (1) evaluating the diversity and spatial variation of the bacterial community composition near Luzon strait; (2) discerning geochemical characteristics of the different water masses and the relationship among the physico-chemical properties of surface water, bacterial distribution and phylogenetic diversity.

MATERIALS AND METHODS

Study stations and water sampling

Sampling was carried out during September 11 to 25 in 2008 on board using the research vessel Shiyan 3. All the 11 study stations were located near the Luzon Strait as listed in Figure 1. The study area is covered with three water masses including the SCS water mass, the Kuroshio water mass and the SCS-Kuroshio transition region water mass. The study area was divided into four transects: Transect A including Station K201 and K205, Transect B including Station K301, K303, K305 and K307, Transect C including Station K402 and K405 and Transect D including Station K502, K505 and K507. Transect A and B were grouped into SCS water mass; Transect C lied at the transition region area and stations of Transect D is located on the eastern side of the Luzon strait. Surface water samples at every station were collected with conductivity-temperature-depth (CDT) (Sea-Bird Electronics, Inc., USA). After collection, 1 L of the surface seawater was filtered with 0.22 µm pore size filters (47mm diameter, Millipore Corp., Bedford, USA) under low (2 m bar) vacuum pressure to collect prokaryotic cells. After filtration, membranes were immediately frozen in liquid nitrogen and then stored at -20°C until DNA extraction in the Lab. Water for nutrient variables measurement were filtered through GF/F glass fiber (0.45 µm, 47 mm diameter, Whatman Japan Limited., Tokyo, Japan) and immediately frozen (-20°C) until analyzed. All the samples were collected in triplicate.

Environmental data analysis

Temperature and salinity of the stations were measured onboard the research vessel using a CTD system (Sea-Bird Electronics, Inc., USA). Seawater samples for analysis were taken using 5 L GO-FLO bottles at surface as described previously (GB12763-91, China; Wang et al., 2008). The water samples were kept at -20°C until analysis. Water samples were analyzed for nitrate (NO_3-N/µmol·L^{-1}), nitrite (NO_2-N/µmol·L^{-1}) and silicate (SiO_4-Si/µmol·L^{-1}) with a SKALAR auto-analyzer (Skalar, Breda, The Netherlands). Ammonium (NH_4-N/µmol·L^{-1}) and phosphorus (PO_4-P/µmol·L^{-1}) were analyzed with methods of oxidization by hypobromite and molybdophosphoric blue, respectively, with a Ultraviolet (UV) 1601 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) according to Wang et al. (2008).

DNA extraction, PCR amplification, and DGGE

The filters were cut into small pieces and transferred into the extraction tubes using ethanol-flamed forceps and surgical scissors and then the community DNA extraction was performed according to the special DNA protocol for the marine bacterial community (Bostrom et al., 2004; Yu et al., 2009). The precipitated DNA was resuspended in 20 µl of TE buffer (10 mM Tris-HCl, 1 mM Na_2 ethylene diamine tetraacetic acid (EDTA), pH 8.0). For PCR purposes, the DNA concentration was measured spectrophotometrically (HITACHI) and adjusted to a concentration of 100 ng μl^{-1}. A 586 bp fragments (*Escherichia coli* numbering position 341-927) of 16S ribosomal deoxyribonucleic acid (rDNA) were amplified by PCR using the primer pair 341F (5'-CCTACGGGAGGCAGCAG-3') and 907R (5'- CCGTCAATTCM TTTGA GTT-3') (Muyzer et al., 1998). A 40 bp GC-clamp (CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G) was added to primer 341F (5') to increase the separation of DNA bands in DGGE gel (Muyzer et al., 1993; Muyzer and Smalla, 1998; Winter et al., 2007). The resulting PCR-fragments had a length of 626 bp due to the GC-clamp. The PCR was

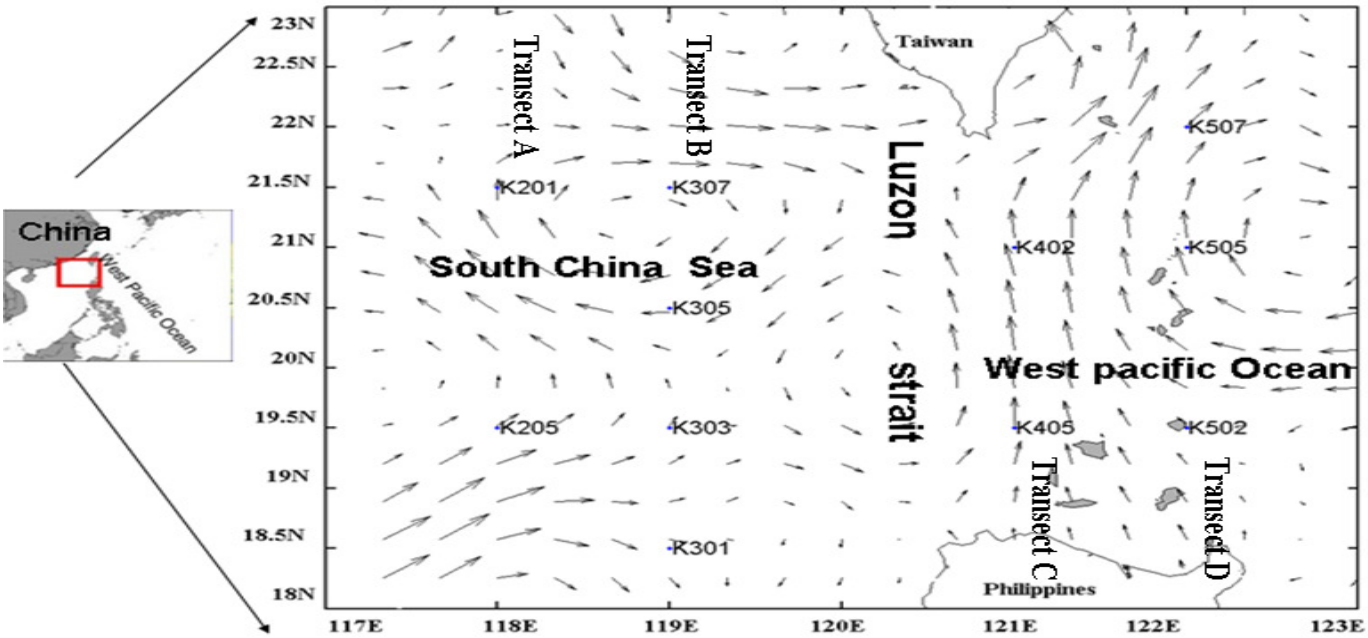

Figure 1. Study area of the 11 sampling stations and the geostrophic current condition near Luzon strait (Transect A, K201 and K205; Transect B, K301, K303, K305 and K307; Transect C, K402 and K405; Transect D, K502, K505 and K507).

performed in 50 µlmreaction containing 200 ng template DNA (negative controls with water), 1×Ex TaqTM Buffer (Takara Shuzo Company Limited Otsu, Japan), 200 µM Deoxyribonucleotide triphosphate (dNTP), 0.4 µM of each primer and 4U Ex polymerase (Takara Shuzo Company Limited Otsu, Japan) in with PTC-2000 thermal cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: an initial denaturation at 95℃ for 5 min, followed by 30 cycles of at 94℃ for 30 s (denaturation); 55℃ for 30 s (annealing), 72℃ for 30 s (extension), and followed by a final extension at 72℃ for 30 min.

The final elongation step was performed at 72℃ for 30 min in order to prevent the formation of artificial double bands in subsequent DGGE analysis (Janse et al., 2004). Each PCR reaction was carried out in triplicate to reduce possible inter-sample PCR variation and pooled together before purifying PCR products. They were concentrated using a PCR purification kit (Takara Shuzo Company Limited Otsu, Japan) according to the manufacturer instructions and obtained a final volume of 40 µl in elution buffer (Takara Shuzo Company Limited, Otsu, Japan). Standard agarose gel electrophoresis was used to size and quantify the PCR fragments. Equal amounts of PCR products (40 µL PCR product with 7 µL loading dye) of different stations were loaded on an acrylamide gel (1mm thick, 6% acrylamide). DGGE was performed with the INGENYphor U-2 apparatus (Ingeny International BV, Goes, The Netherlands). The 100% denaturating solution was composed of 7 M urea and 40% formamide (v/v). Electrophoresis was performed with 8% polyacrylamide gel in a set of glass plates (1 mm x 28.2 cm x 20.2 cm) with gradient of denaturant from 50 to 80% in 1x Tris–acetate– ethylene diamine tetraacetic acid (TAE) buffer (40 mM Tris, 40 mM acetic acid, 1 mM EDTA; pH8.0). DGGE were performed at 60℃ during 17 h at a constant voltage of 100 V. After electrophoresis, DGGE gels were stained with ethidium bromide and visualized under UV light using AlphaImager imaging system (Alpha Innotech Corp., San Leandro, CA, USA). DGGE digital images were analyzed by gel documentation system, Gel Doc 2000, Quantity-One 4.5.2 (Bio-Rad, Hercules, CA, USA) to generate a densitometric profile.

Sequencing and phylogenetic analysis of DGGE profile

Distinct DNA bands were excised from the gel, resuspended in 20 µl of TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) and left at 4℃ overnight to elute DNA. The supernatant after centrifugation (12000 rpm, 5 min, 4℃) was used as a template and was reamplified as previously described. The PCR products were loaded again in a DGGE gel to confirm the position of the bands. Products of the same mobility were purified, ligated into PMD18-T cloning vector, and subsequently transformed into *E. coli* DH5α according to the manufacturer's instructions (Takara Shuzo Company Limited Otsu, Japan). Positive recombinants were then submitted for sequencing using an ABI3730 DNA Sequencer (USA) with M13 primer at the Shanghai Invitrogen Biotech Company Limited. The sequences obtained were analysed against sequences in the Ribosomal Database Project (RDP) for Chimera check and then using the Classifier tool (Maidak et al., 1999) and against GenBank sequences using the program Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1997). Phylogenetic trees of 16S rDNA partial sequences were generated using the neighbor-joining algorithms in Mega IV software (Tamura et al., 2007). The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al., 2004) and were in the units of the number of base substitutions per site.The level of support for the phylogenies derived from neighbor-joining analysis was gauged by 1000 bootstrap replicates (Saitou and Nei, 1987).

Analyses of DGGE patterns and statistical analysis

DGGE profiling of bacterial community were digitized after average background subtraction for the entire gel. The peak areas of the fingerprint patterns were used to indicate the intensities. Bands with

Table1. Physicochemical parameters of the water samples (value are given as mean, n=3).

Site	Temperature (ºC)	Salinty (‰)	NO_3-N (umol/L)	NO_2-N (umol/L)	NH_3-N (umol/L)	SiO_4-Si (umol/L)	PO_4-P (umol/L)	Species (N)
K201	29.19	33.36	3.54	0.09	1.63	1.48	0.35	30
K205	29.66	33.53	3.31	0.04	0.42	0.35	0.24	28
K301	30.03	33.49	7.35	0.03	0.64	1.00	0.46	30
K303	29.92	33.56	6.94	0.03	0.38	2.45	1.32	32
K305	29.31	33.59	5.90	0.02	0.21	1.16	0.35	33
K307	29.04	33.48	4.91	0.02	1.10	1.00	0.24	29
K402	29.32	33.23	11.66	0.02	0.60	1.97	0.35	33
K405	29.07	33.47	12.35	0.03	0.55	1.82	0.24	34
K502	28.98	34.15	9.63	0.02	0.08	0.23	0.46	34
K505	28.58	34.17	10.09	0.03	1.02	0.23	0.46	35
K507	28.67	33.81	11.66	0.02	0.24	2.13	1.32	30

a relative intensity of less than 0.5% of the sum of all band intensities were discarded. Band position and intensity data for each station were exported to an excel spreadsheet prior to further statistical analyses. The diversity indices of the DGGE digital image were analyzed by gel documentation system, Glyko Bandscan (Glyko Biomedical Limited, USA) according to Zhang et al. (2009). Similarity analysis was performed using data produced from the DGGE profiles of 16S rDNA. The images were analyzed for the number of bands per sample (presence versus absence) and transformed into a data matrix representing presence and absence of bands by ones and zeros, respectively (Schäfer and Muyzer, 2001).

The cluster was determined by unweighted pair-group method with arithmetic mean (UPGMA), using the MultiVariate Statistical Package (MVSP) v3.1 (GeoMem, Blairgowrie, UK). To best explore the available data, we conducted the multivariate statistical analysis by RDA (Redundancy Analysis) using the CANOCO 4.5 for Windows (Biometris, The Netherlands). RDA is a direct extension of multiple regressions for the modeling of multivariate environmental species data, which can relate the quantitative changes in bacterial community to *in situ* environmental variables directly.

Temperature, salinity, nitrate, nitrite, silicate, ammonium and phosphorus were included for analysis in this study. Detrended correspondence analysis (DCA) was firstly done to decide between linear or unimodal response model for these bacterial species data. The length of the first DCA ordination axis was 2.325 for bacterial species data, so we chose RDA to ordinate the spatial bacterial community to the measured environmental parameters of the seawater (Lepš and Šmilauer, 2003; Zhang et al., 2011).

Data transformation was carried out to the data matrices containing relative band intensities and the explanatory variables in order to approximate normal distribution. It was carried out using abundant DGGE bands (the relative intensity exceeded 0.5%) together with environmental variables (Muckian et al., 2007).

The statistical significance (at the 5% level) of relationship between species data (deduced from DGGE profiles) and environmental variables were assessed using the Monte Carlo test on 499 random permutations (P<0.01 and P<0.05) to test the null hypothesis that bacterial profiles were unrelated to environmental variables (Zhang et al., 2009).

Nucleotide sequence accession numbers

All the sequences obtained in this study have been deposited in GenBank™ with the accession numbers of GU196284–GU196294 and GU196296-GU196302.

RESULTS

Environmental characteristics of the study area

The basic physicochemical parameters of the study area investigated were summarized in Table 1. The variation of these variables of different transects was obvious. Compared to other transects, higher concentrations of nutrients [phosphate (PO_4^{3-}), nitrite, ammonium, nitrate (NO_2^-+NO_3^-+NH_4^-) and silicate (SiO_4^-)] were detected in Transect C during the cruise (Table 1). The average value of the concentration of Transect C was 14.78 umol/L while for the Transects A, B and D, the concentration of the nutrients was 5.73, 8.91 and 12.53 umol/L, respectively. The lowest value of temperature was observed in Transect D with the average of 28.74°C while all stations of Transect D showed the highest value of salinity with the maximum value of 34.17‰. The geostrophic current analysis (Figure 1) showed that path of surface Kuroshio near Luzon strait was a route from northeast of Luzon to southwest of Taiwan with a branching loop into SCS and then went eastward along the continental slope of northern SCS.

DGGE profile and phylogenetic positions of 16S rDNA gene sequences

A total of 343 detectable bands in 105 different positions were detected in the DGGE gel. The range of the bands per station was from 24 to 35 (mean 31), indicating a high diverse bacterial assemblage in the surface water on both sides of Luzon strait. The most intense DGGE bands were excised and then sequenced (Figure.2), which were verified by DGGE three times to ensure a

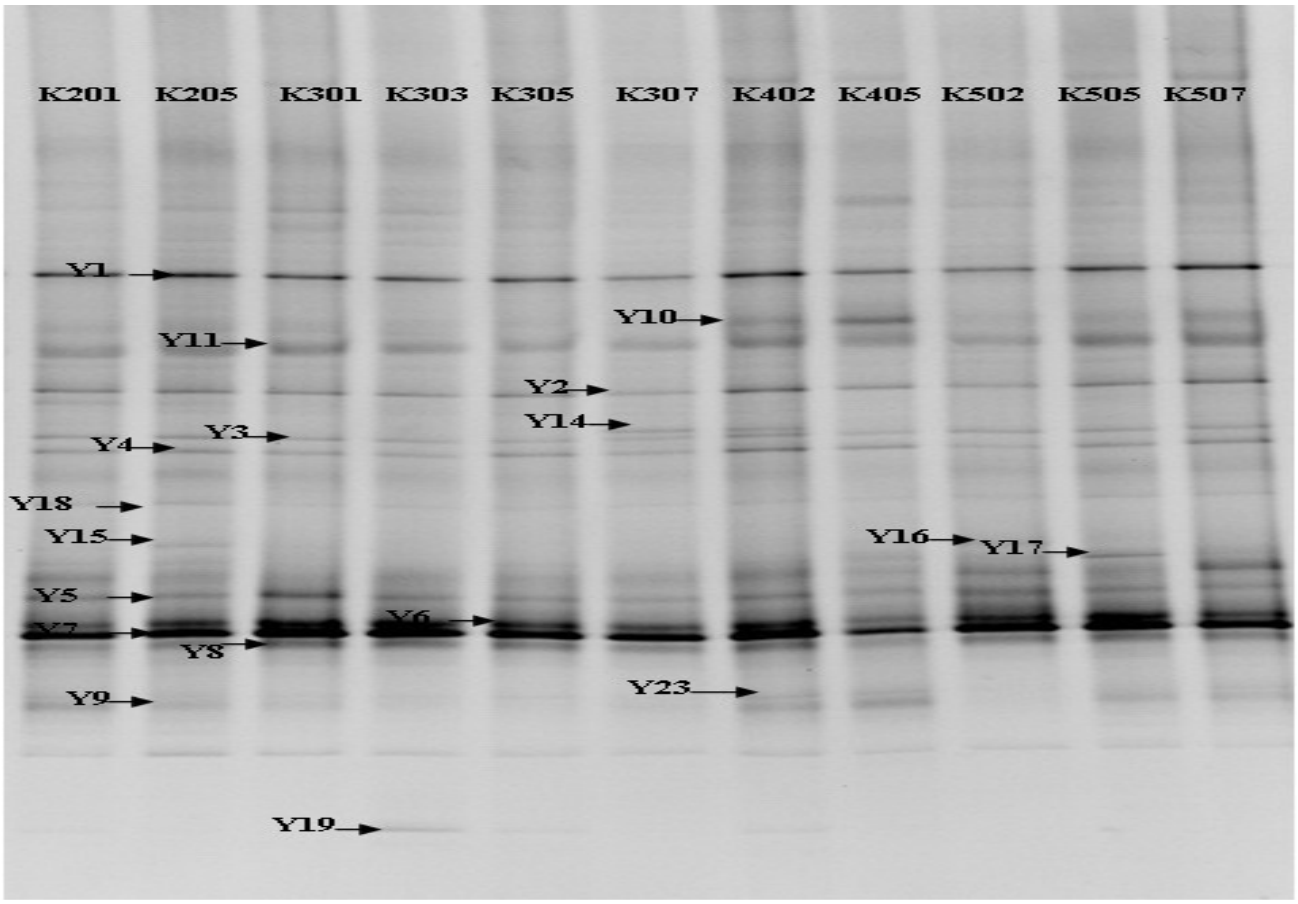

Figure 2. DGGE patterns of amplified 16S r DNA gene fragments of bacterial communities at the sampling sites near the Luzon Strait and sequences of DGGE bands labeled were excised and sequenced.

single band at the same location. A total of predominate 18 DGGE bands were successfully sequenced. Sequencing analysis that the predominant phylotypes represented the excised DGGE bands are summarized in Table 2 and Figure 5. The bacteria identified from surface ocean water were closely related to one of the following groups: *Cyanobacteria* (33.3%, six sequences), *Alphaprotebacteria* (22.2%, four sequences), *Gammaprotebacteria* (16.7%, three sequences), *Deltaprotebacteria* (5.6%, one sequence) and *Antinobacteria* (22.2%, four sequences). Most of the sequences obtained from the surface water were similar to 16S rDNA gene sequences reported from uncultured organisms present in marine environmental samples such as seawater, Delaware Bay and oligotrophic region of the Kuroshio current (Table 2). The percentage similarity of clones and its closest blast hits ranged from 94 to 100%, respectively. In addition, one phylotypes (Y5) with 94%, one (Y23) with 95% sequence and two sequences (Y9 and Y17) with 97% identity to the closest related sequence in GenBank were obtained, possibly representing novel bacterial lineages (accounting for 22.2% of total bacterial sequences).

The *Proteobacteria* group including the eight phylotypes were detected frequently, with four phylotypes (Y4, Y11, Y17 and Y19) belonging to the subphylum *Alphaproteobacteria* (Table 2). The high detection frequency of these *Alphaproteobacteria* phylotypes suggested that they were an important component of the bacterial community in the Northern SCS. Subphylum *Deltaproteobacteria* (Y18) could be obtained in almost every station. Two phylotype (Y2 and Y5) of four *Actinobacteria*-like sequences was present in all the stations, while the two other sequences (Y16 and Y23) were limited to the Transect A. *Cyanbacteria* (Y3, Y6, Y7, Y8, Y10 and Y15) were distributed almost at all the surface water of the investigated stations near Luzon strait, which indicated that it is widespread in the Northern SCS. The phylogenetic analysis showed that all the members of *Cyanobacterium* investigated were *Prochlorococcus*-like phylotypes (Table. 2).

Cluster analysis of bacterial community structure

As shown in Figure 3, hierarchical cluster analysis was

Table 2. Closest matches to excised and sequenced 16S rDNA-derived from the DGGE profile.

Phylogenetic group	Band	Accession number	Database match with accession number in parentheses	Origin	Identity %
Cyanobacteria	Y3	GU196286	Uncultured *Prochlorococcus* sp. (FJ903247)	oligotrophic region of the Kuroshio Current	99
	Y6	GU196289	Uncultured bacterium (GU062041)	South China Sea	99
	Y7	GU196290	Uncultured *Prochlorococcus* sp. (FJ903215)	oligotrophic region of the Kuroshio Current	99
	Y8	GU196291	Uncultured *Prochlorococcus* sp. (EU361165)	ocean water collected from Hawaii Ocean	99
	Y10	GU196293	Uncultured *cyaobacterium* (GQ349213)	Saanich Inlet, 120 m depth	99
	Y15	GU196297	Uncultured *Prochlorococcus* sp. clone 031806#34 (FJ903269)	oligotrophic region of the Kuroshio Current	100
Alpha *protebacteria*	Y4	GU196287	Uncultured bacterium clone Reef_O14 (GU119424)	reef water	99
	Y11	GU196294	Uncultured bacterium (EU804279)	250 miles from Panama City	99
	Y17	GU196299	Uncultured bacterium clone N412B_51 (GU940922)	South China Sea	97
	Y19	GU196301	Uncultured *alphaproteobacterium* clone ARTE9_164 (GU230201)	coastal water	99
Gamma *protebacteria*	Y1	GU196284	*Alteromonas* sp. 56(HQ188656.)	surface seawater sample of South Korea in the East Sea	99
	Y9	GU196292	Uncultured bacterium (EF573831)	site S25 near Coco's Island	97
	Y14	GU196296	Uncultured bacterium clone 1NTfc10_H11 (GQ413501)	coral-associated bacterial community colony 10 from near-1 site at time point 22 days	99
Delta *proteobacteria*	Y8	GU196291	Uncultured *Prochlorococcus* sp. (EU361165)	ocean water collected from Hawaii Ocean Time-series Station ALOHA	99
Antinobacteria	Y2	GU196285	Uncultured bacterium (GU206795)	South China Sea	100
	Y5	GU196288	Uncultured bacterium (EU800735)	Delaware Bay, NJ	94
	Y16	GU196298	Uncultured bacterium (GU206794)	South China Sea water	99
	Y23	GU196302	Uncultured bacterium (GU206795)	South China Sea	95

employed to group stations having similar bacterial community structure. UPGMA clustering using our DGGE data revealed banding patterns among the four transects were different. The results of cluster analysis indicate that all stations could be divided into two groups and then further subdivided into four subgroups (Figure 3). All stations (except Station K307) of the same transect tended to cluster together. The bacterial community structure of Transects A and B were more similar and grouped together in the dendrogram. Station K307 had a similar bacterial community composition with K402 and K405 rather than grouping with the subgroup of Station K301, K303 and K305. As a whole, the station on

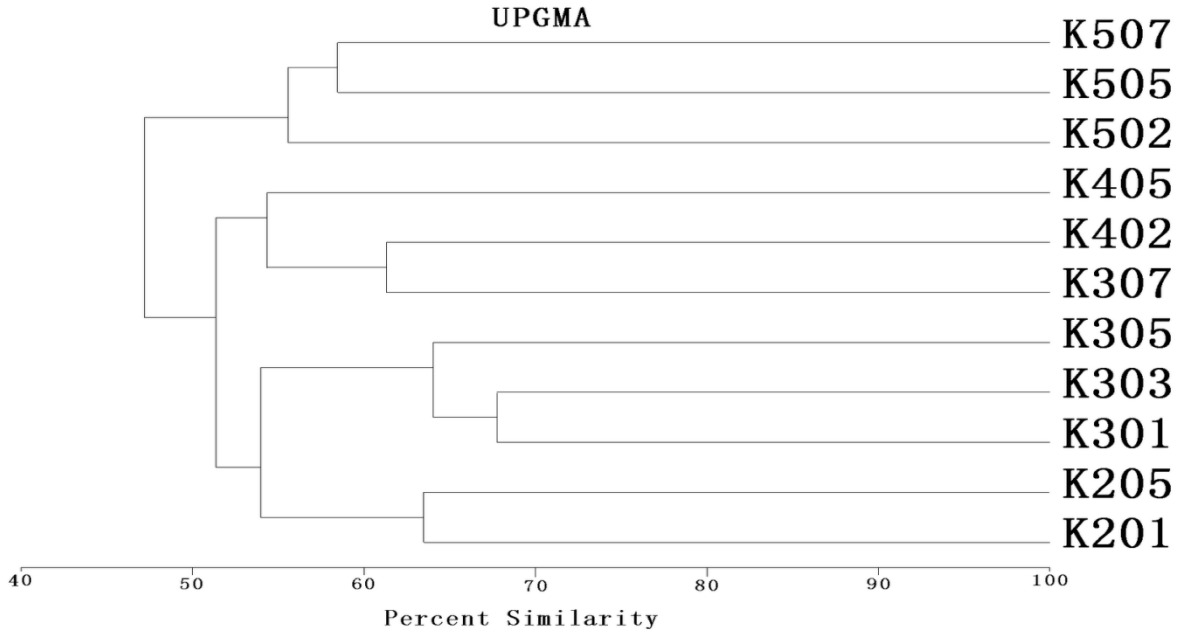

Figure 3. UPMGA dendrogram constructed from the similarity matching data obtained from the DGGE profiles of 16S r DNA gene partial sequences amplified from the sample of the stations located near the Luzon strait.

the same side of the Luzon strait had higher similarity of the bacterial community and the Transect C had more similar bacterial species with Transects A and B in common. The cluster analysis results were in agreement with the geotrophic current condition (Figure 1).

Relationship between environmental variables and bacterial community structure

RDA established the DGGE bands characterizing the bacterial community structures in all sampling stations (Figure 4). The arrow vectors for the environmental variables in each plot represent their significance in the distribution of bacteria at the sampling stations. Eigenvalues for the first two multivariate axes were 0.191 and 0.116, respectively, explaining 19.1 to 30.7% of the total variance in each RDA. The sum of all canonical eigenvalues was 1.000. Monte-Carlo significance tests indicated that both axes explained a significant proportion of the variation in the data. According to Monte Carlo test, the *P*-value of the significance of first canonical axis and significance of all canonical axes was 0.004 and 0.012, respectively. This indicates that the environmental variables may play important role in spatial variation of the bacterial community composition near Luzon strait. The first canonical axis was significantly correlated with salinity and nitrate (P=0.014 and 0.038, respectively), indicating that the first axis mainly showed the gradient of salinity and nitrate near Luzon strait. The second axis was correlated with temperature (P=0.004), which revealed the changes in spatial bacterial community composition mainly caused by the temperature. RDA confirmed the clear separation of stations according to environmental factors that all the stations can be divided into two groups on the basis of geographic location: the eastern and western stations.

DISCUSSION

There were two major water masses near the Luzon strait, which were Kuroshio and SCS water mass. Liang et al. (2008) found that Kuroshio flowed consistently into the SCS and most of the intruded water made a loop and flowed out of the northern Luzon strait, but a branch of the Kuroshio intruded into the SCS. As a result, the physical and chemical properties of ocean water were different from west to east on the two sides of Luzon strait. The cluster analysis showed that bacterial community structures difference was obvious and all the stations formed two groups on the basis of the geographic location. All the stations were further divided into four subgroups on the basis of longitude. According to Figure 1, the transition region are more influenced by the SCS water rather than the Kuroshio Current and these stations of Transect C had more similar bacterial community composition with the station located on the western side of the Luzon strait (Transects A and B). RDA analysis confirmed the cluster analysis result that all stations were separated into two parts: Circle A included the stations located on the left side of the Luzon strait

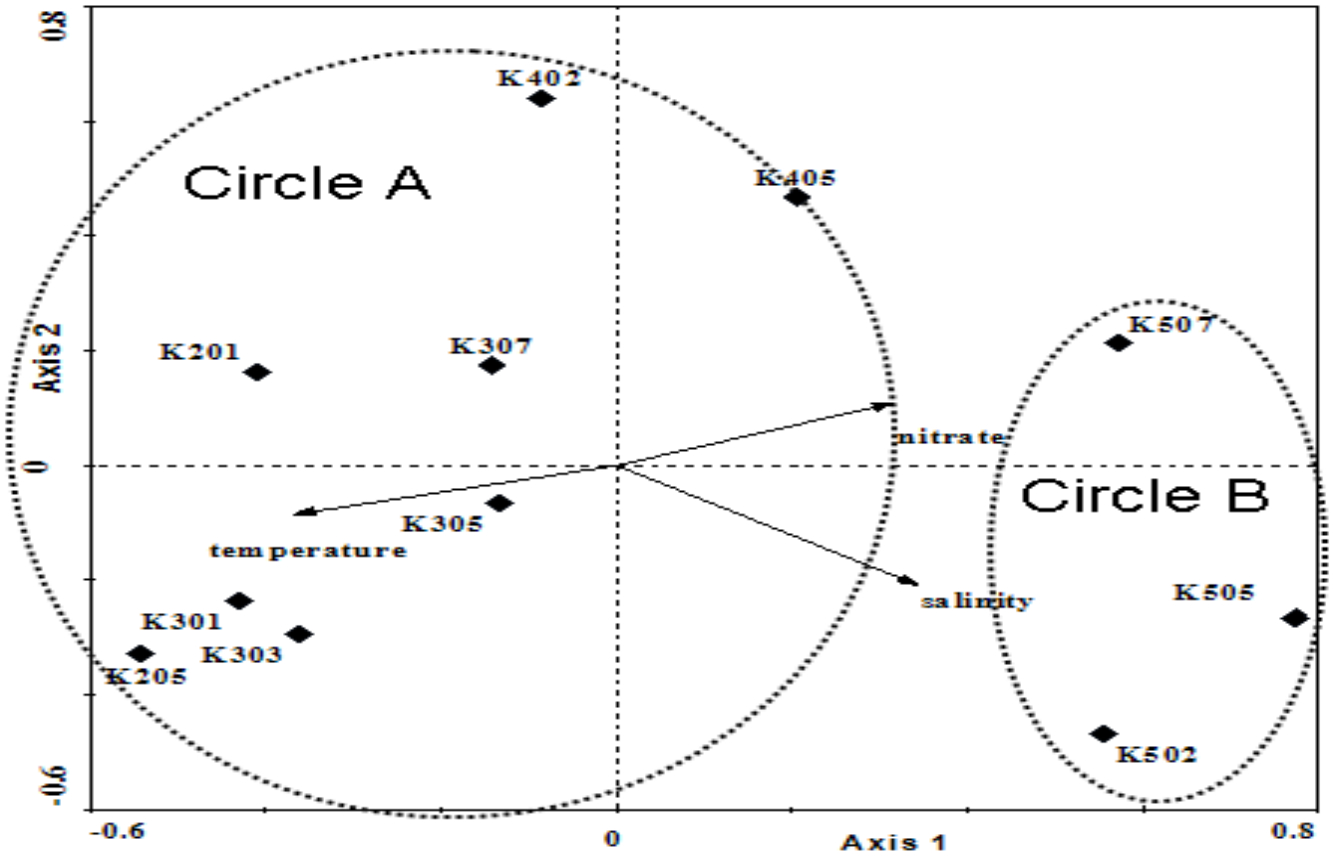

Figure 4. RDA bioplot diagram of DGGE bands data (samples indicated using sample stations location code) and environmental variables (represented by arrows).

and Circle B contained stations located on the right side of the Luzon strait (Figure 4).

This was in agreement with the results of Li et al. (1998), which indicated that the Kuroshio current was most limited to east of 121°E. Several bacterial species or phylotypes can be detected almost at all the stations, such as the species Y3, Y4, Y6 and Y7, while Y14 and Y23 were only found at the station K307 and K402. Species Y15 can be only detected from Transects A and B. Bacterial communities of these stations were characterized by the occurrence of Species Y16 and Y17. Due to mix of the two water masses, the stations of Transect C exhibited the highest species diversity of all the investigated stations. The species mean value of Transect C was 33.5 (Table 2), while for the Transects A, B and D, the values were 29, 31 and 33, respectively. Sequence analysis revealed that most of the phylotypes were typical marine uncultured bacteria and predominant bacteria came from three major phyla, *Proteobacteria*, *Actinobacteria*, and *Cyanobacteria*. This result is in agreement with previous assessments of microbial diversity in surface ocean waters in the northwest Atlantic Ocean (Erin et al., 2009; Giovannoni and Rappe`, 2000; Pommier et al., 2007). In addition, Sipura et al. (2005) also found that Alpha and Gamma-*Proteobacteria*, *Actinobacteria* and *Cyabacteria* were predominant in the brackish-water Archipelago Sea. Feng et al. (2009) reported that clone sequences related to *Alphaproteobacteria* were the most abundant in the Changjiang estuary and coastal area of the East China Sea. In addition to water column, bacterial diversity of SCS sediment exhibited greater diversity while they were consisted of *Proteobacteria*, *Planctomycetes, Actinobacteria*, *Firmicutes*, *Chloroflexi*, *Acidobacteria*, *candidate division OP8*, *Bacterioidetes/Chlorobi* and *Verrucomicrobia* (Li et al., 2008). *Cyanobacteria* accounted for a large proportion of the detected species, such as species Y3, Y6, Y7, Y8, Y10 and Y15, which was consistent with the previous studies in SCS (Dong et al., 2008; Ma et al., 2009; Ren et al., 2006).

They can both photosynthesize and fix nitrogen and contribute a lot to the primary production, especially in the pelagic area (Hoffman, 1999; Karl et al., 1997). Previous reports showed they had specific abilities to degrade both aromatic hydrocarbons and xenobiotics (El-Bestawy et al., 2007). However, during our investigation,

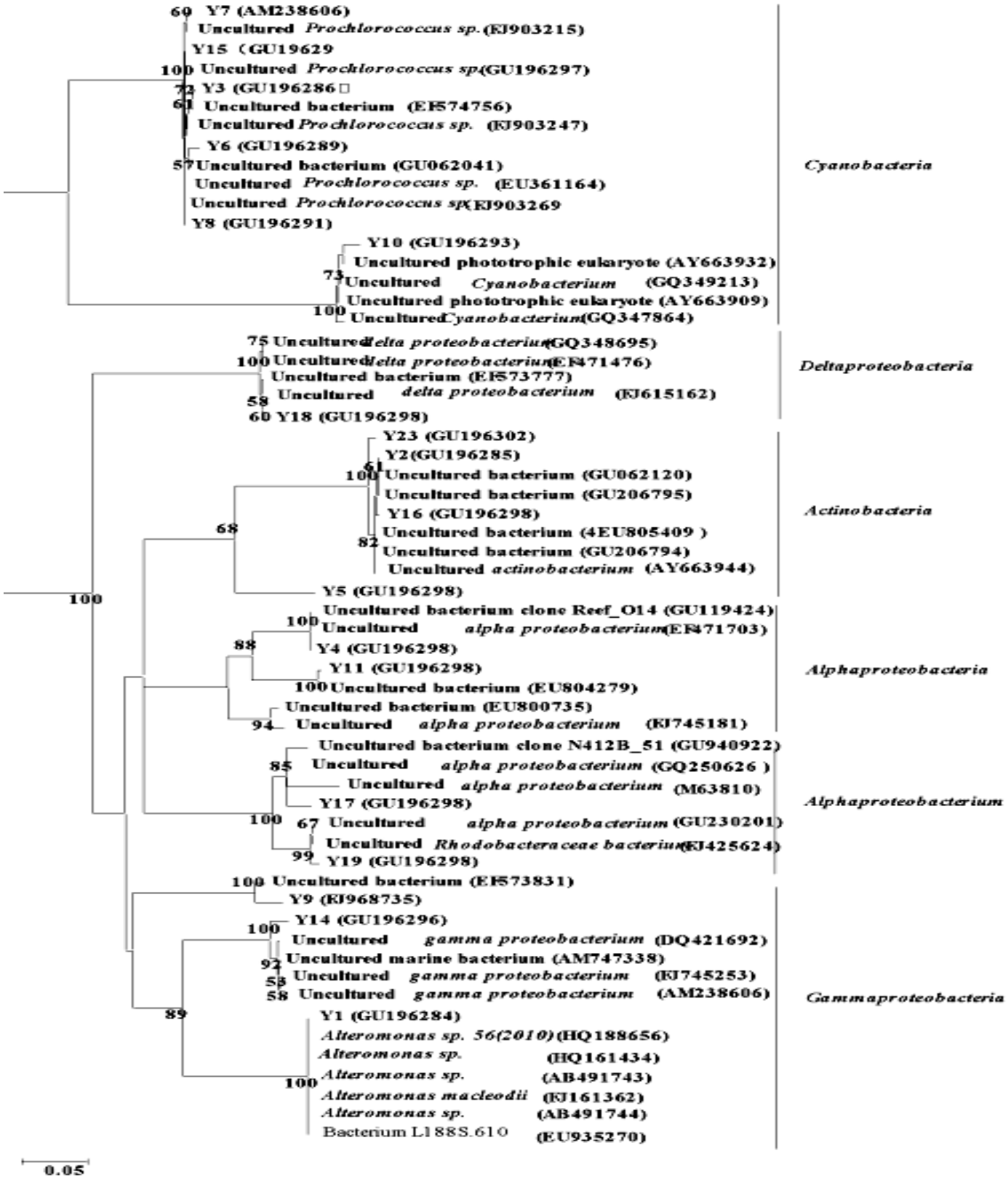

Figure 5. Unrooted phylogenetic tree based on partial 16S rDNA sequences representing the respective DGGE bands in Figure 2. Bootstrap analysis was based on 1000 replicates. Bootstrap values from distance analysis are depicted. Bootstrap values less than 50% were not shown. Scale indicates 5% sequence divergence.

only the one type *Prochloroccus* was discovered and they were related to the high-light-adapted ecotype (HL) *Prochlorococcus* strain from SCS and seawater near Coco's Island (Table 2). Ma et al. (2009) also found that the distribution of Cyanobacteria was primarily limited to the photic zone and the genetics of *Prochlorococcus* showed a good relationship with depth adaptation in SCS. In this study, eight phylotypes were identified as *Proteobacteria*. *Alphaproteobacteria* was the predominant distributed bacteria species in the surface water near the Luzon strait. Six phylotypes (33.3%) belonged to the phylum *Alphaproteobacteria*. That is in agreement with the observation that *Alphaproteobacteria* were important members of bacterioplankton in open oceans and coastal environments (Field et al., 1997; Fuhrman et al., 1993; Giovannoni et al., 1990). Fuhrman and Hagström (2008) also pointed out that the *Alphaproteobacteria* was generally abundant and had high phylogenetic diversity in the ocean ecosystem. Species Y9 showed only 97% percent similarity to the closest matches in the National Center for Biotechnology Information (NCBI), which indicate that it may be a new species. For the Gamma *proteobacteria* observed in our investigation, the 16S rDNA sequences of Species Y1 showed high similarity (99%) to those reported previously (Table 2).

However, Species Y14 had low similarity (95%) to those reported previously in the NCBI Genbank, indicating a new genotype. Only one species of the *Deltaproteobacteria* was detected in all the water samples. *Protebacteria* played important roles in the biogeochemical cycles, such as nitrogen fixation (Zehr et al., 1998), sulfate-reduction (Schoenborn et al., 2001) and degradation of polycyclic aromatic hydrocarbons (PAH) (Jiménez et al., 2011). Three *Antinobacteria*-like phytotypes were also detected. The bands Y5 and Y23 only had 94 and 95% similarity with the closest previously reported sequences in NCBI, respectively, which indicate that they were possible new species. *Actinobacteria* have been well studied because they produced two-thirds of the microbially derived antibiotics. It has long been used as a source of commercially useful enzymes and therapeutically useful bioactive molecules (Berdy, 1989; Jiang et al., 2007). The RDA ordination showed the significant environmental variables for the bacterial species distribution near the Luzon temperature, salinity and nitrate. Temperature and salinity were also most suitable hydrographic properties in defining water masses and studying their distribution. Nitrogen concentration may have a direct effect on the bacterioplankton composition or through indirect effect on the changes in biomass and composition of phytoplankton (Haukka et al., 2006). Different concentration of nutrient can also be used to differentiate the water masses. Hence, it is feasible to use the RDA ordination to investigate the water masses near Luzon strait. Previous studies had showed that salinity was a very important environmental factor for the distribution of the bacterial species (Bouvier and del Giorgio, 2002; Crump et al. 1999; Kataoka et al., 2009). Yan et al. (2008) detected that the bacterial community composition was significantly correlated with the variables of nitrate, dissolved oxygen and silicate in the stream and Zeng et al. (2009) found total nitrogen, ammonia and severed pH as significant environmental factors affecting the microbial community structure. Several studies also found that other environmental parameters, such as ChlA and turbidity could affect the bacterial community (Kataoka et al., 2009; Yannarell and Triplett, 2005). These results indicate that different significant environmental parameters control bacterial community in different ecosystem.

Different bacteria also responded quite differently to the same condition, such as in the study of the effect of nutrient enrichment on bacterioplankton biomass community composition in mesocosms in the Archipelago Sea (Sipura et al., 2005). The results show that *Verrucomicrobia* had their highest relative intensity in the control treatment and their lowest in the higher nutrient addition treatment, whereas most *Synechococcus*-related bands exhibited their lowest relative intensity in the lower nutrient addition treatment (Sipura et al., 2005). Gao et al. (2005) reported that Beta and Gamma *proteobacteria* preferred high nitrate/nitrite concentrations while *Alphaproteobacteria* was more abundant in environment where nitrate/nitrite concentrations were low.

Conclusions

The bacterial communities near the Luzon strait were mainly composed of *Protebacteria*, *Antinobacteria* and *Cyanobacteria*. *Alphaprotebacteria* were a dominant group. Spatial variation of the bacterial community composition was obviously seen from the DGGE profile. The similarity cluster analysis showed that all the stations were divided into two groups: (I) Stations located on the western side of the Luzon strait and (II) Stations located on the eastern side of the Luzon strait. They could be further subdivided into four groups on the basis of the longtitude.

The variation of community composition was significantly related to the physico-chemical characteristics, including temperature, salinity and nitrate (P=0.004, P=0.014 and P=0.038, respectively, P<0.05). Analysis of spatial variation of the bacterial community composition in this area by combination of the molecular techniques with statistical analysis may provide the basis for the further investigation of the functional bacterial community near the Luzon strait.

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Abbreviations

SCS, South China Sea; **DGGE**, denaturing gradient gel electrophoresis; **PCR**, polymerase chain reaction; **RDA,** redundancy analysis; **CTD**, conductivity-temperature-depth; **DCA**, detrended correspondence analysis; **PAH,** polycyclic aromatic hydrocarbons; **NCBI,** national center for biotechnology information; **MVSP,** multivariate statistical package; **UPGMA,** unweighted pair-group method with arithmetic mean; **BLAST,** basic local alignment search tool; **TAE,** Tris–acetate– ethylene diamine tetraacetic acid; **dNTP,** deoxyribonucleotide triphosphate; **EDTA,** ethylene diamine tetraacetic acid; **rDNA,** ribosomal deoxyribonucleic acid; **UV,** ultraviolet; **DNA,** deoxyribonucleic acid; **PCR-DGGE,** polymerase chain reaction-denaturing gradient gel electrophoresis.

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