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Spatial variation of bacterial community structure of the Northern South China Sea in relation to water chemistry

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Abstract Spatial distribution, diversity and composition of bacterial communities of the northern South China Sea (SCS) surface water and the relationship with the in situ environmental chemistry were investigated. Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) was used to investigate the bacterial community structure. The DGGE gel showed that each sample harbored a distinct bacterial community structure and spatial variations of bacterial community composition among all samples were obviously. A total of 17 intensive bands were excised and the sequence analysis of these DGGE bands revealed that Proteobacteria were the dominant bacterial group of surface water in the north part of SCS. Results of the taxonomic analysis showed that the communities consisted of Proteobacteria (α-subdivision, β-subdivision, γ-subdivision), Actinobacteria, Cyanobacteria, Bacteroidetes and Firmicutes. Unweighted pair group method with arithmetic averages clustering of the sampling stations indicated that all stations were classified mainly based on geographical proximity. Canonical correspondence analysis (CCA) was employed to further investigate the relationships between DGGE band pattern and the environmental variables and the first two CCA ordination axes suggested that the structure of the bacterial community was significantly correlated with the variables of nitrate ($F = 1.24$, $P < 0.05$).

Keywords The Northern South China Sea · Bacterial community structure · Water chemistry · PCR-DGGE · Canonical correspondence analysis

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Introduction

Marine bacteria are considered to play important roles in the ecological system. That is due to the fact that they are producers and consumers of the marine food web, where photosynthetic and organotrophic microorganisms collectively contribute a lot towards bringing the habitat into an equilibrium state, which is important for the conservation of other life forms. Community diversity and species persistence are significant characteristics of the natural habitats (Al-Sayed et al. 2005). Activities of some bacteria also affect the nutrient cycle. Many molecular biological methods are now available to phylogenetically describe complex communities of unculturable bacteria (Amann et al. 1995) and molecular techniques based on rDNA genes obtained from natural environment can provide new insights into the diversity of marine microbial composition. Cloning and sequencing of rDNA have been a very useful method for describing the compositions of marine bacterial community structure (Giovannoni et al. 1990), however, analysis of clone libraries is very time-consuming and inefficient when many different samples are analyzed at

the same time, especially the temporal or spatial changes of microbial composition changes along environmental gradients were studied. So another technique that allows processing of many samples simultaneously was needed for such studies. Denaturing gradient gel electrophoresis (DGGE), which was introduced by Muyzer et al. (1993) as a new culture-independent genetic fingerprinting technique, now is one of the most widely used approaches that are used in investigating several patterns of distribution of marine bacterial assemblages (Moeseneder et al. 1999; Murray et al. 1996; Riemann et al. 1999; Schauer et al. 2000). Several factors have been described to influence bacterial community in the aquatic ecosystem (Vrede 2005), such as phosphorus (P), nitrogen (N) and organic carbon (OC), especially N and P in the marine ecosystem. It is in that in oligotrophic marine ecosystem, primary productivity is often limited by N or P (Ahmed et al. 2008; Thingstad et al. 1998). Researches on the relationships between the bacterioplankton community structure and water chemistry could yield many useful information for understanding the process of biogeochemical cycling of nutrient elements. Nowadays, many multivariate statistical analysis methods have been used to investigate the relationships between bacterial community composition and environmental parameters (Sapp et al. 2007; Zhang et al. 2009), such as canonical correspondence analysis (CCA). It has been proven to be sensitive and efficient in evaluating the bacterial community structure and the environmental factors (Mouser et al. 2005). These studies demonstrated that total phosphorus, organic matter, salinity, pH and factors associated with nitrogenous compounds played significant roles in the bacterial community composition.

The South China Sea (SCS) is the second largest marginal sea in the world with an area of 3.5×10^6 km^2 and an average depth of about 1,350 m. It locates between the equator and 23.8 N and from 99.1 E to 121.1 E and is characterized by tropical and subtropical climate. The northern part of SCS represents typical oligotrophic characteristics, with significant environmental gradients due to the discharge of the Pearl River and is also influenced by many types of physical forcing, such as mesoscale eddies, monsoon, upwelling, Kuroshio Current and so on (Chen et al. 2006; Han 1998). Detailed experimental study has been made to investigate the water chemistry in the northern SCS (Xu et al. 2008; Long et al. 2006), however, little information is known in regarding to the relationships between environmental parameters and the structure of bacterial communities in the northern part of SCS, so the PCR-DGGE technique has been performed for the following aims.

The objectives in the paper are as follows: (a) to have an overview of bacterioplankton community structure of the surface water of the northern part of SCS and (b) to investigate the relationship between variations of the bacterial community structure in relation to the in situ water chemistry.

Materials and methods

Study stations and water sampling

The cruise was conducted in late August and early September 2008. Eighteen stations were occupied during the cruise. The study sites were located in the northern part of

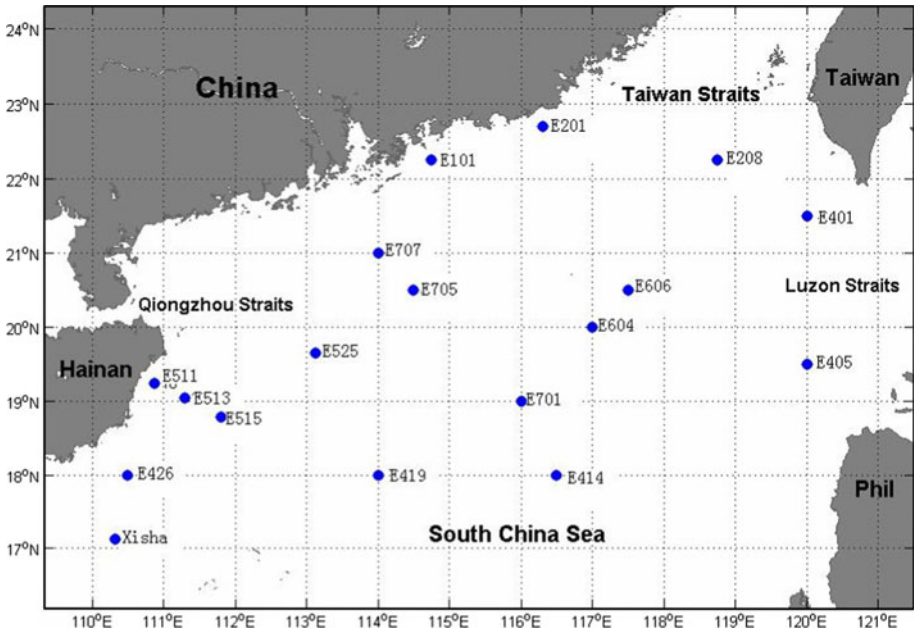

Fig. 1 Distribution of the sampling stations in the northern part of the SCS

SCS as listed in Fig. 1. All the samples were taken by conductivity–temperature–depth (CTD; Sea-bird). After each sample collection, one liter of the surface seawater was filtered onto 0.22 μm, 47 mm diameter Durapore (Millipore, Bedford, USA) filters under low (2 m bar) vacuum pressure without prescreening to remove large particles. After filtration, membranes were immediately frozen in liquid nitrogen and then stored at −20 °C until DNA extraction in the lab. Water for physicochemical parameters measurement were filtered through GF/F glass fiber (0.7 μm, 47 mm diameter, Whatman, Tokyo, Japan) and immediately frozen (−20 °C) until analyzed.

Environmental data

Temperature and salinity of all stations were measured with a CTD. Seawater samples analysis for nutrients and dissolved oxygen (DO/mg L^{-1}) were taken using 5-L GO-FLO bottles at surface according to the protocols of "The specialties for oceanography survey" (GB12763-91, China) (Wang et al. 2006, 2008, 2011). Water samples were analyzed for nitrate (NO_3-N/μmol L^{-1}), nitrite (NO_2-N/μmol L^{-1}) and silicate (SiO_3-Si/μmol L^{-1}) with a SKALAR auto-analyzer (Skalar Analytical B.V. SanPlus, Holand). Ammonium (NH_4-N/μmol L^{-1}), phosphorus (PO_4-P/μmol L^{-1}) were analyzed with methods of oxidized by hypobromite and molybdophosphoric blue. Dissolved oxygen (DO/mg L^{-1}) was determined using Winkler titrations (Pai et al. 1993). The detection limits of NO_3-N, NH_4-N, NO_2-N, PO_4-P and SiO_4-Si were 0.05, 0.03, 0.02, 0.02, 0.45 μmol L^{-1}, respectively according to the specification for marine monitoring (GB: 17378.6-1998).

DNA extraction, PCR amplification, and DGGE

Total community DNA extraction was performed according to the special DNA protocol for the marine bacterioplankton community (Boström et al. 2004; Yu et al. 2009). Fragments of 16S rDNA (position 341–907) were amplified by PCR using the primer set 341F (5′-CCTACGGGAGGCAGCAG-3′) and 907R (5′-CCGTCAATTC(A/C)TTT(A/G) AGTT-3′). A 40 bp GC-clamp (cgcccgccgcgcgcg gcgggcggggcgggggca cgggggg) was added to primer 341F (5′) to increase the separation efficiency of DNA bands in DGGE gel (Muyzer et al. 1993; Muyzer and Smalla 1998).

The PCR was carried out in 50 μL reaction system with mixture of 200 ng DNA template (negative controls with water), 1× Ex Taq TM Buffer, 200 μM dNTPs, 0.4 μM of each primer and 2U Ex polymerase. The Taq polymerase, dNTPs and PCR buffer were purchased from TaKaRa (TaKaRa, Japan). Thermal cycling was carried out under the following conditions: initial denaturation at 95 °C for 5 min, followed by 30 cycles of primer annealing at 55 °C for 30 s, chain extension for 30 s at 72 °C, denaturation for 30 s at 94 °C and a final extension at 72 °C for 10 min. Each PCR was carried out in triplicate to reduce possible inter-sample PCR variations and pooled them together before loading on DGGE gel.

PCR products were separated using an INGENY phorU-2 (Ingeny International BV, the Netherlands) DGGE system. Equal amounts of PCR products of different stations were loaded on an acrylamide gel (1 mm thick, 6 % acrylamide) with a 45–70 % linear gradient of denaturant (100 % denaturant consisted of 7 M urea and 40 % (v/v) formamide). Electrophoresis was performed with 1× Tris–acetate–EDTA (TAE) buffer at 60 °C at 200 V for 10 min, followed by 100 V for 18 h. After electrophoresis, DGGE gels were stained with ethidium bromide and visualized under UV light using AlphaImager imaging system (Alpha innotech, USA). DGGE digital images were analyzed by gel documentation system, Gel Doc 2000, Quantity-One 4.5.2 (Bio-Rad, USA) to generate a densitometric profile. The peak areas of the fingerprint patterns were used to indicate the intensities. Bands with a relative intensity of less than 0.5 % of the sum of all band intensities were discarded.

Sequencing and phylogenetic analysis

Distinct DNA bands were excised from the gel and transferred to 20 μL PCR quality water and left at 4 °C overnight to elute DNA. The supernatant after centrifugation (12,000 rpm, 5 min, 4 °C) 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 using the PCR Purification Kit (Bioteck, China) and subsequently cloned into *Escherichia coli* DH5α cells, using the PMD18-T cloning vector kit according to the manufacturer's instructions (Takara, Japan). Positive recombinants were then submitted for sequencing using an ABI3730 DNA Sequencer (USA) with M13 primer at the Shanghai Invitrogen Biotech Co. Ltd. Nucleotide sequences were compared with those in the GenBank database by BLAST algorithm for tentative identifications. Phylogenetic trees of 16S rDNA partial sequences were generated using the neighbor-joining algorithms in Mega IV software. The level of support for the phylogenies derived from neighbor-joining analysis was gauged by 1,000 bootstrap replicates.

Diversity and statistical analyses

The diversity and dissimilarity indices were analyzed according to the DGGE banding profiles. The

Table 1 Physicochemical parameters of surface water in northern part of the SCS

Station	Temperature (°C)	Salinity (‰)	NO_3-N (μmol L^{-1})	NO_2-N (μmol L^{-1})	NH_3-N (μmol L^{-1})	PO_4-P (μmol L^{-1})	SiO_4-Si (μmol L^{-1})	DO (mg L^{-1})
E101	27.61	31.35	2.05	0.09	3.74	1.75	6.32	6.82
E201	25.93	32.77	2.33	0.02	0.02	0.62	6.66	6.30
E208	29.25	33.31	2.39	0.16	1.68	0.40	0.23	5.37
E401	29.20	33.51	4.36	0.04	0.76	0.51	2.08	5.51
E405	28.98	33.29	3.78	0.02	0.24	0.51	1.19	5.46
E414	29.38	33.60	8.25	0.02	0.02	0.06	2.42	6.23
E419	29.97	33.19	0.27	0.03	1.25	0.51	1.07	6.76
E426	30.24	33.07	9.10	0.02	0.29	1.19	2.09	6.54
Xisha	30.12	33.08	7.28	0.03	0.87	0.40	0.90	4.43
E511	29.52	33.60	7.77	0.02	0.02	0.17	0.22	6.45
E513	29.90	33.61	2.10	0.01	0.52	0.40	0.22	6.39
E515	29.00	33.63	10.52	0.05	0.68	0.79	0.22	6.57
E525	29.77	33.44	5.09	0.04	1.17	0.51	0.22	6.60
E701	29.82	33.67	4.37	0.03	0.93	0.17	0.39	6.39
E705	28.70	33.46	1.37	0.03	0.77	0.28	0.39	4.12
E707	27.05	33.68	5.56	0.02	0.31	0.06	0.05	6.45
E604	29.55	33.60	2.09	0.02	0.02	0.17	2.25	6.36
E606	28.94	33.41	2.80	0.04	1.36	0.62	1.92	6.17

Shannon–Weaver index of bacterial diversity H', was calculated by using the following equation (Shannon and Weaver 1963), $H' = -\sum_{i=1}^{S} Pi \ln P$, Pi is the importance probability of the bands in a track. It was calculated from $Pi = ni/N$, where ni is the area of a peak and N is the sum of all peak areas in the densitometric curve. Hierarchical cluster analysis was performed using data produced from the DGGE profiles of 16S rDNA. 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 CCA using the CANOCO 4.5 for Windows, which can relate the quantitative changes of bacterial community to water quality directly. Six environmental variables related with water quality, which includes nitrate, nitrite, silicate, ammonium, phosphorus and DO, were included. Data matrices containing relative band intensities and the explanatory variables were also log $(x + 1)$ transformed in order to obtain approximate normal distribution. The statistical significance (at the 5 % level) of relationships between species data (DGGE profiles) and environmental variables were assessed using the Monte Carlo test on 999 random permutations to test the null hypothesis that bacterial profiles were unrelated to environmental variables.

Results

Environmental parameters and DGGE patterns analysis

The basic physicochemical parameters of the surface water samples were shown in Table 1. The variations of these parameters at different sampling stations were obviously. For instance, station E515 had highest the concentration of nitrate, with the value of 10.53 μmol/L, which is more than 38 times of station E419. Station E101 had the highest concentration of the ammonium, phosphorus and DO. The concentration of silicate of all stations ranged from the 0.05 to 6.66 μmol/L.

The bacterial community structures of different stations are shown in Fig. 1. It can be seen that the banding patterns among all the samples were distinct. DGGE gel analysis yielded a total of 290 detectable bands in 76 different positions. The range of the bands per sample was from 12 to 21 (mean 16), indicating a diverse bacterial assemblage in the surface water of the northern SCS. The indices of H', reflecting the structural diversity of the bacterial community, were calculated on the basis of the number and relative intensities of bands on the gel track (Fig. 1).Based on Table 2, the lowest Shannon–Weaver indice was detected at station E208, while the highest Shannon–weaver indice was found at station E419. The range of index value varied from 2.47 to 3.03 (mean 2.74).

Table 2 Composition and the Shannon diversity index of bacterial community

Station	DGGE bands	Shannon–Weaver diversity (H')
E101	17	2.826
E201	14	2.611
E208	17	2.786
E401	14	2.611
E405	12	2.470
E414	15	2.693
E419	21	3.027
E426	18	2.854
Xisha	17	2.825
E511	12	2.463
E513	17	2.817
E515	20	2.956
E525	18	2.856
E701	17	2.822
E705	16	2.760
E707	17	2.811
E604	15	2.698
E606	13	2.552

Community structure

Hierarchical cluster analysis was employed to group stations having similar bacterial community structure (Fig. 2). It was obvious to see that stations were classified primarily based on geographical features (except Station E606). All stations were clustered into six subgroups. For example, station E101 and E201 clustered together in subgroup VI and they both located in the vicinity of continental margin. Stations E511, 513, 515 locating near the Hainan Island were in the same group III. In the east part of the SCS, there were stations E208 and E401 in the same cluster V showing more similarity in the bacterial composition. Stations E419, Xisha, E701 and E705 were in the same cluster II. From Fig. 2, it can be seen that station E606 vastly differed from others stations.

Relationship between environmental variables and bacterial community structure

Correspondence analysis established the DGGE bands characterizing the bacterial community structures in all the sampling stations (Fig. 1), which was carried out using abundant DGGE bands (the relative intensity exceeded 0.5 %) together with environmental variables (Muckian et al. 2007). The significant relationships between environmental variables and canonical axes were analyzed according to Monte Carlo permutation test (999 permutations) using Canoco program ($P < 0.01$ and $P < 0.05$). The CCA of the 16S rDNA DGGE data explained 35.1 % and 27.1 % of the variation in the first two axes (Fig. 3). Eigenvalues for the first two multivariate axes were 0.317 and 0.245, respectively and the sum of all canonical eigenvalues was 1.242. And species environment correlations for both axes were more than 0.93, indicating that bacteria were strongly correlated with environmental

Table 3 Closest matches to excised and sequenced 16S rDNA-derived DGGE bands

Band	Accession No.	Database match closest with accession no. in parentheses	Origin	Identity (%)
GH1	GU206794	Uncultured bacterium (AB307989)	Seawater, Japan, western North Pacific	100
GH2	GU206795	Uncultured bacterium (GU119453)	Reef water	99
GH3	GU206796	Uncultured bacterium (EU802627)	Environmental sample Northeast of Colon, Panamá	99
GH4	GU206797	Uncultured bacterium (GU062151)	Seawater, South China Sea	99
GH5	GU206798	Uncultured bacterium (GU119424)	Reef water	99
GH6	GU206799	Uncultured bacterium (GU119340)	Reef water	100
GH7	GU206800	Uncultured bacterium (EF575134)	Environmental sample site S25 near Coco's Island	100
GH8	GU206801	Uncultured bacterium (EF574984)	Environmental sample site S25 near Coco's Island	96
GH9	GU206802	Uncultured bacterium (GU119389)	Reef water	99
GH10	GU206803	Uncultured bacterium (GU062049)	South China Sea	99
GH11	GU206804	Uncultured alpha proteobacterium (AY663927)	East China Sea	99
GH12	GU206805	*Labrenzia* sp. (GQ495021)	Cultivation of Karlodinium micrum	99
GH13	GU206806	*Oceanobacter* sp. (AB435651)	Seawater, Indonesia: Jakarta, Pari island	96
GH14	GU206807	Uncultured Synechococcus sp. (EU361321)	Ocean water collected from Hawaii Ocean	98
GH15	GU206808	Uncultured bacterium (GU119216)	Reef water	99
GH16	GU206809	Uncultured marine bacterium (AM747367)	Beach surface water	99
GH17	GU206810	Uncultured bacterium (FJ957841)	Spacecraft associated clean room	93

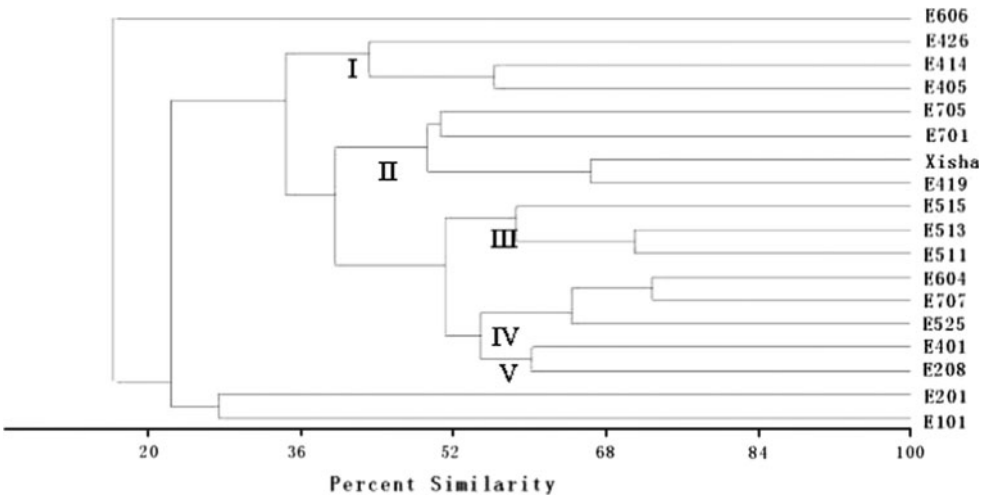

Fig. 2 Cluster analysis of DGGE gel pattern showing the amplified 16S r DNA gene fragments from the surface water of the northern SCS

factors. Based on CCA, axis 1 was positively correlated with nitrate, ammonia, silicate and DO and correlations coefficient values between them were 0.7046, 0.3979, 0.4683 and 0.2370, respectively. Conversely, axis 2 had a negative correlation with nitrate, with the value of 0.2825. Monte-Carlo significance tests indicated that both axes explained a significant proportion of the variation in the data and according to Monte Carlo analysis, only nitrate ($F = 1.34$, $P = 0.034$) showed a significant correlation with general community structure.

Based on Fig. 3, it was clear that most of the selected dominant bacterial phylotypes had a negative correlation with nitrate, which were distributed on the left side of the biplot, including the bacterial phylotypes GH2, GH3, GH4, GH5, GH6, GH 8, GH10, GH11, GH12 and GH17 (see numbers and positions in Fig. 4). Bacterial phylotypes GH6 and GH10 were positively correlated with ammonia and bacterial phylotypes GH14 showed a clear positive correlation with ammonia, silicate and DO. Furthermore, phylotypes r23, r59 and r72 were positively correlated with nitrate.

Phylogenetic positions of 16S rDNA gene sequences

The most intense DGGE bands from surface water samples were excised and then sequenced (Fig. 4), which were verified three times by DGGE to ensure a single band at the same location. A total of 17 DGGE bands were successfully sequenced. Sequence analysis of the predominant phylotypes that represented the excised DGGE bands was summarized in Table 3. All the sequences obtained in this study had been assigned to the GenBank nucleic acid sequence database under accession numbers GU206794–GU206810. According to Table 3, sequence analysis showed that most of the sequences obtained from the surface water were uncultured organisms present in environmental samples from sources such as reef water, seawater, beach surface water and so on. The percentage similarity of clones and its closest blast hits ranged from 93 to 100 %, respectively. As shown in Fig. 5, Band GH3, GH5, GH6, GH15 and GH16 were observed in almost all the sampling stations. Six bands (GH3, GH5, GH8, GH11, GH12 and GH15) were Alpha-Protebacteria-like sequences and GH1, GH2 and GH6 were Actinobacteria-like sequences. Band GH17 and GH9 related to Firmicutes-like and Beta-protebacteria-like sequence, respectively. Two bands (GH10 and GH3) fell into the Gamma-Protebacteria cluster. Band GH4, GH14 and GH16 belonged to Synechococcus order in the Cyanobacteria class. Band GH17 was affiliated with phylum Bacteroidetes.

Discussion

Due to the inability to cultivate all the bacteria from environmental samples in laboratory media, it is very useful to employ the culture-independent method to study the diversity of the surface water (Amann et al. 1995). And previous works have demonstrated that DGGE was a good molecular technique to analyze the bacterial community in seawater (Haller et al. 2000; Fromin et al. 2002; Sapp et al. 2007; Tanaka et al. 2008). Extraction of bacterial DNA of interest is very crucial in evaluating the true conditions of the sampling stations, therefore optimizing the DNA extraction methods is very important. We compared several methods in community DNA extraction and chose the best one. Since potential biases inherent in the PCR-DGGE method have been reported previously (Suzuki and Giovannoni 1996), so the DNA extraction of all samples were subjected to the same procedure and PCR amplification using low-stringency annealing temperature and small number of amplification cycles in this study to avoid the PCR bias as much as possible (Zeng et al. 2009).

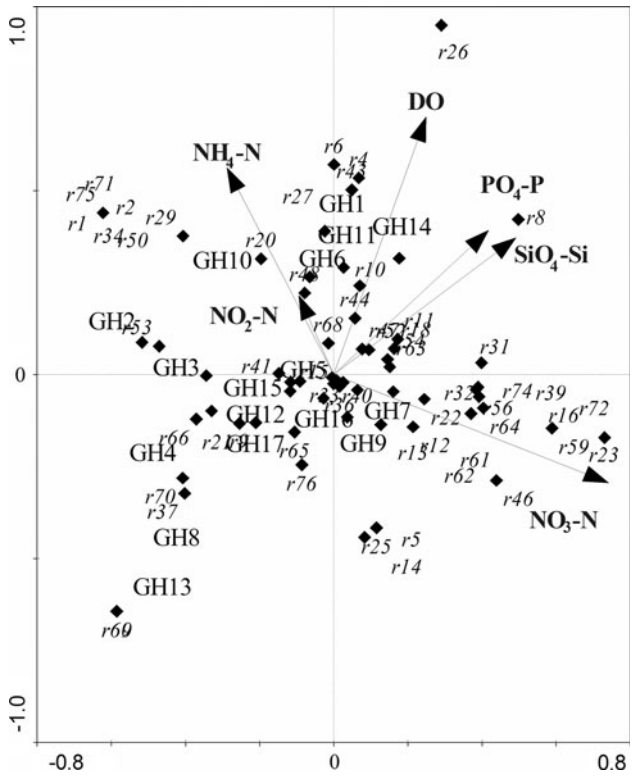

Fig. 3 Canonical correspondence analysis (CCA) ordination diagram of DGGE data, with chemical factors as arrows and individual abundant bacteria

Figure 4 showed all bacterial community patterns on the lanes. The predominant of sequenced bands extracted from the DGGE profiles of this study were identified as Alpha, Beta and Gamma Proteobacteria. Other bands were identified within the phylum of Cyanobacteria, Actinobacteria, Bacteroidetes and Firmicutes, accounting for less percentage. However, in the Yellow Sea Cold Water Mass, phylogenetic analysis showed that bacterial phylotypes were only made up by Proteobacteria (γ-Proteobacteria and δ-Proteobacteria) and Bactericides (Liu et al. 2008a). Phylotypes of cloned bands of the hypoxia of the Changjiang estuary showed that bacterial community were mainly consisted of Proteobacteria, Bacteroides, Firmicutes and Cyanobacteria (Liu et al. 2008b). Riemann et al. (1999) reported that the dominant bacteria of the Arabian Sea were Alpha and Delta proteobacteria, and no bands related to Gamma proteobacteria or Cytophaga-Flavobacterium-Bacteroides (CFB) have been detected. The investigation by Haller et al. (2000) indicated that the sequenced bands of DGGE profiles were typical marine isolates of both alpha-subclass and gamma-subclass of the Proteobacteria and the CFB branch in the Western Mediterranean Sea. The differences of bacterial community composition among all the stations were clear. This may be the interaction results of the area-specific physical and chemical environment. However, Phylum Proteobacteria was present in all the stations and they were a common bacterial species in the marine environment, especially the Alpha and Gamma subclasses, which had already been confirmed by Pinhassi et al. (1997). In our study, α-Proteobacteria account for 29.4 % of all the sequenced obtained. This result was consistent with study by Acinas et al. (1999). It has been assumed that the Alpha subclass of the class Proteobacteria played an important role

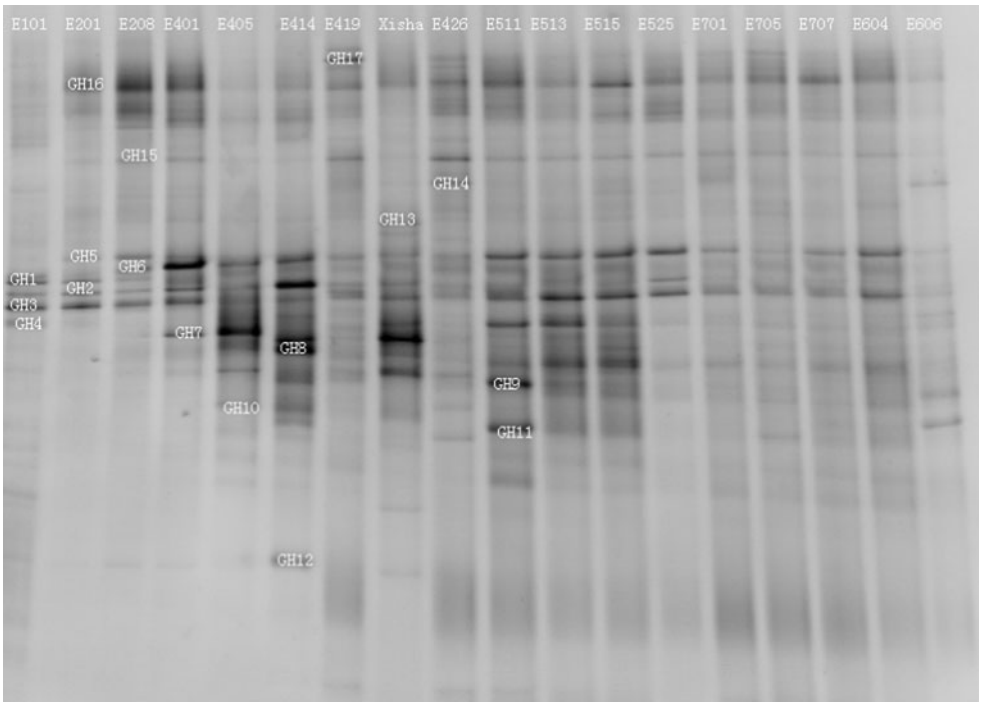

Fig. 4 DGGE fingerprints of PCR-amplified 16S rDNA of bacteria from the seawater samples. Lanes 1–18 correspond to the sampling stations and the sequenced band can been seen with the label from GH1 to GH17

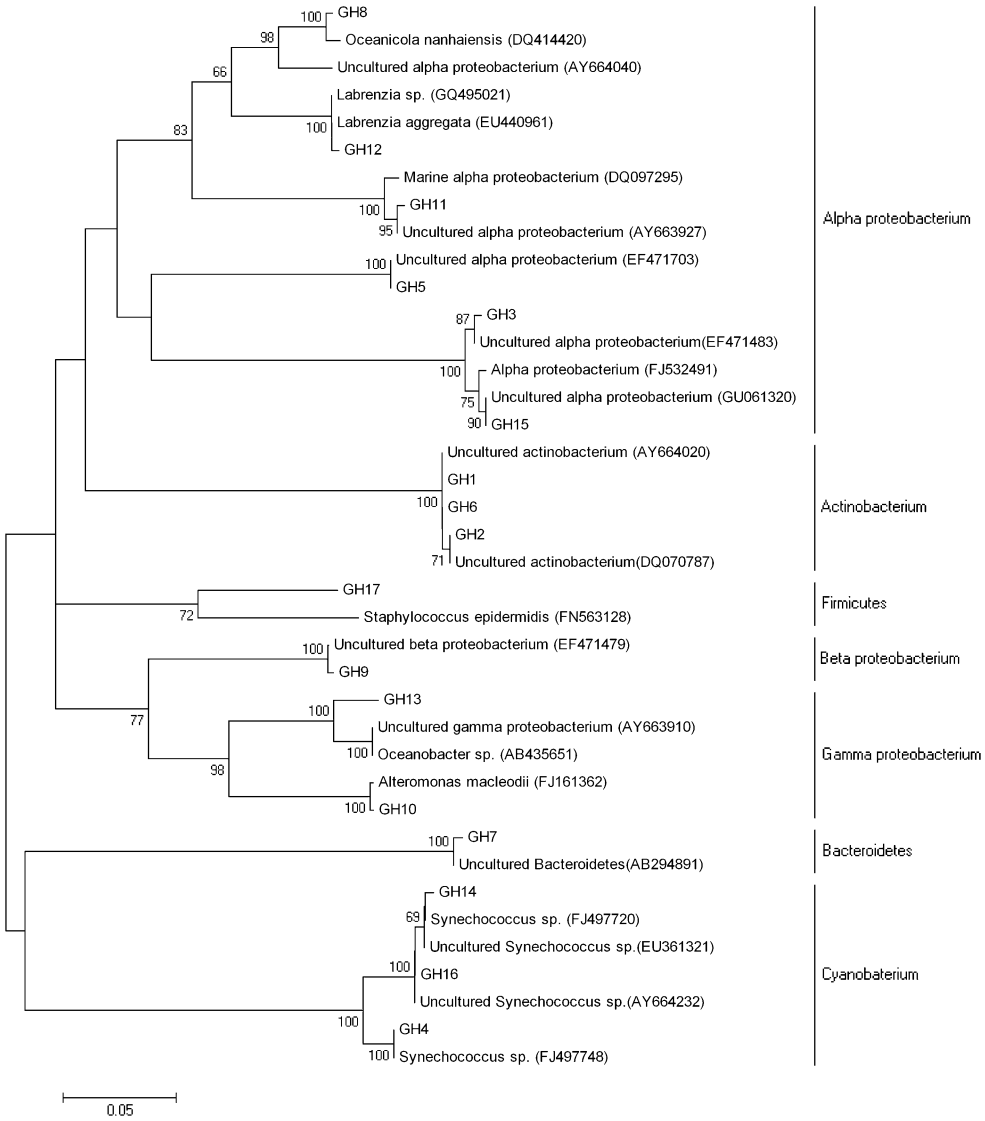

Fig. 5 Unrooted phylogenetic tree based on partial 16S rDNA sequences representing the respective DGGE bands in Fig. 4. Bootstrap analysis was based on 1,000 replicates. Bootstrap values from distance analysis are depicted. Bootstrap values less than 50 % were not shown. Scale indicates 5 % sequence divergence

in the consumption of dissolved organic matter and the total phosphorus removal (Ahmed et al. 2008; Pinhassi et al. 2004). Furthermore, one of our obtained sequences belonged to the β-Proteobacteria subgroup, two to the γ-subgroup. Previous studies indicated that the β-Proteobacteria was a common group in freshwater systems (Brachvogel et al. 2001; Schweitzer et al. 2001). Alpha- and gamma-Proteobacteria were more frequently found to be the dominant groups in higher salinity environments (Henriques et al. 2006). Phylum Cyanobacteria was also an important component of plankton community. Through our study, we only obtained the phenotype of Synechococcus, which confirmed the investigation that Synechococcus were distributed abundantly in the surface water (Ma et al. 2004). Due to their ability to fix nitrogen, Synechococcus contributed substantially to the input of new nitrogen into the oligotrophic environment (Ohlendieck et al. 2000). Furthermore, some of the heterotrophic bacteria affiliated with subclass of α- and γ-Proteobacteria were analyzed based on phylogenetic analysis of the *nif*H gene and the results showed that they were also capable to fix nitrogen in the marine environment (Bess et al. 2007). They both played important roles in the nitrogen cycle and contributed a lot to the primary productivity of the marine ecosystem. Studies on the Actinobacteria of the SCS have already been done on the sponges and soil sediment, both culturable and unculturable (Jiang et al. 2007; Li et al. 2006; Tian et al. 2009). They has become an important material for searching novel natural products as a resource for new drugs (Ward and Bora 2006). Actinobacteria-like sequences were detected in stations E101, E201, E208 and E401, which indicated that SCS harbor rich antibacterial assemblages.

Cluster analysis of bacterial communities showed that there were distinct discrepancies among all the sampling stations. For instance, stations located near the Hainan Island had more similar bacterial community. Based on

Fig. 2, stations E511, E513 and E515 being in the same cluster. That may due to the reason that they were under the effect of the QiongDong upwelling and shared more similar geographical features. Likewise, stations E101 and E201 lied in coastal area and were affected by the Guangdong coastal Current and outflows of the Pearl River, where the water was well mixed and nutrients were high all year around (Lin et al. 2011). Figure 2 also showed that these two stations clustered together. Stations E701 and E705 located near the Dongsha Island while stations E419 and Xisha were near the Xisha Island. The Island effect was the possible cause that they possessed similar bacterial community structure and it also could explain why stations E525, E604 and E707 were in the same subgroup and station E208 clustered together with E401. Stations E414, E405 and E426 were all sited a certain distance away from the continent and they had more bacterial species in common. The bacterial composition of station E606 differed from other stations. That may be the cause of the intensive water mass movement in this area (Han 1998).

The relationship between the bacterioplankton community and the water environmental parameters has been investigated in other aquatic environments (Bai et al. 2009; Gao et al. 2005; Yan et al. 2008; Zeng et al. 2009). Analysis result suggested that the bacterial community composition was significantly correlated with the variables of nitrate, DO and silicate (Yan et al. 2008). Zeng et al. (2009) previously reported that total nitrogen, ammonia and pH severed as significant environmental factors affecting the microbial community. In this study, CCA analysis revealed that nitrate accounted for a significant amount of the variability in the bacterial community structure. This indicated that available nitrate could affect the community structure of the surface water, and this was similar to Gao et al.'s result (2005). Possible reason was interaction result of chemical factors and physical conditions of the aquatic ecosystem influenced bacterial community to different extent. Our result further confirmed that β- and γ-Proteobacteria preferred high nitrate/nitrite concentrations while α-Proteobacteria were more abundant in environments where nitrate/nitrite concentrations were low (Gao et al. 2005). 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). This result was consistent with that variations in the bacterial community were affected not only by abiotic factors but also by the phytoplankton community (Rooney-Varga et al. 2005; Sapp et al. 2007). Nitrogen is thought to be one of the leading pollutants of lakes and estuaries and also had been estimated as the water eutrophication indicator (Parry 1998). Nitrates enter the ocean through rainfall and river discharge and benefit plankton in oligotrophic ocean due to that primary productivity of which is limited by the available nitrate. We only did the qualitative analysis of the bacterial community of the stations, so future research should focus on the bacterial community composition quantitatively. It could been testified through nutrient addition and isotope calibration experiments to investigate to what extent nutrients affect the structure of microbial communities plus their cycling activities.

Conclusion

This study employed the PCR-DGGE and multivariate statistical analysis to investigate the bacterial community structure of the northern SCS. Results showed the spatial variation of bacterial community structure of surface water of the northern SCS was obvious. Taxonomic analysis indicated that the communities mainly consist of Proteobacteria (α-subdivision, β-subdivision, γ-subdivision), Actinobacteria, Cyanobacteria, Bacteroidetes and Firmicutes. It can be seen from the cluster analysis that stations possessing similar geographical proximity tended to have similar community structure. Furthermore, we also investigated the effects of chemical factors on the bacterial community composition and found that nitrate was the primary driver of bacterial community change.

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