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Original Research

Vertical Variations of Bacterial Community Composition in South China Sea Determined by DGGE Fingerprinting and Multivariate Analysis

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Abstract

Vertical variations of bacterial community composition in the South China Sea was investigated on 18 September 2009 by denaturing gradient gel electrophoresis (DGGE) and analyzed by multivariate analysis. Twenty-seven sequences retrieved from DGGE bands fell into five groups based on BLAST analysis. The dominant bacteria were *Cyanobacteria* (35.7%) and *Proteobacteria* (39.2%). The DGGE profile showed *Proteobacteria* mostly obtained from samples from the deeper layers while sequences related to *Cyanobacteria* only existed in the euphotic layer. Other phylogenetic groups have been identified as *Firmicutes* (10.7%), *Actinobacteria* (7.1%), and *Deinococcus-Thermus* (3.6%). The unweighted pair group method with arithmetic mean has been employed to cluster the samples, and results indicated that all samples tended to group together on the basis of depth and could be further subclassified into two subgroups: Group I (including samples from 0 m, 50 m, 75 m, 100 m, and 150 m) and Group II (including samples from 200 m, 400 m, 500 m, 600 m, 700 m, and 900 m). Canonical correspondence analysis revealed the temperature was the most significant factor in determining the vertical distribution of the bacterial community ($P=0.018$, $P<0.05$).

Keywords: bacterial community, vertical distribution, South China Sea, cluster analysis, canonical correspondence analysis, PCR-DGGE

Introduction

Marine bacteria, both as producer and consumer, are thought to be an important component of food webs, and they also play a crucial role in the geochemical cycling of elements such as carbon, nitrogen, and sulfur [1]. Due to the high abundance and high growth rates of bacteria, changes in their biomass and community composition would affect ecosystem functions [2, 3]. In addition, the growth of bacteria in the ocean is often limited by such environmental factors as temperature, salinity, nitrate, nitrite, and so on [4-6]. Martiny et al. [7] reported that spatial patterns of microorganisms reflect those of larger organisms.

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Therefore, it is necessary to investigate bacterial community composition and environmental factors. Culture-independent surveys are essential methods to investigate the bacterial phylotypes, because only about 1% of bacteria in the natural environment can be cultured with currently available culture-based methods [8]. Cloning libraries have been a useful method applied to reveal enormous biodiversity in marine environments [9], but analysis of clone libraries is time-consuming and inefficient when many different samples are analyzed at the same time. Muyzer et al. [10] first introduced a new fingerprinting technique denaturing gradient gel electrophoresis (DGGE) as a method for profiling complex environmental microbial communities, which has been proven to be an efficient diagnostic tool and yielded a high-resolution band profile for further investigation [6, 11-14]. Multivariate analysis of PCR-DGGE fingerprints is an approach that is the most commonly studied tool used for calculating the impact of environmental factors on microbial community composition in ecosystems. High-resolution DNA fingerprinting combined with multivariate analysis has been used to investigate variability in the marine microbial community structure in aquatic ecosystems [14].

The South China Sea (SCS) with its deep basin 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 consists of many ecosystems, including mangrove, coral reef, and seagrass, so that the biological diversity there is remarkable [15]. Spatial variation of bacterial community composition in marine sediment and ocean water has been investigated in SCS; however, the vertical pattern of microbial communities in relation to the environmental factors has seldom been carried out in the SCS [5, 6, 16-18].

Consequently, we used polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) followed by DNA sequence analysis to perform a survey of the bacterial community structures in SCS [19]. The aims of this investigation were:

(a) To have an overview of the vertical profile of bacterioplankton community composition in the northern SCS
(b) To investigate the significant factors driving the community variations.

The results of this study will contribute to facilitating better understanding of the vertical bacterial community structure and elucidate the relationship between community variation and environmental factors in SCS.

Materials and Methods

Study Area and Sampling Methods

Samples were collected during an open cruise of R/V Shiyan 3 in September 2009. Temperature and salinity of the Xisha Station were measured onboard the research vessel using a CTD system (Sea-Bird Electronics, Inc., USA). The seawater samples were collected in triplicate. Samples for analysis were taken using 5 L GOFLO bottles in triplicate as described previously [20, 21]. One liter of seawater samples was filtered to collect bacterial cells. After filtration, the membranes were immediately frozen in liquid nitrogen and stored at -20ºC until DNA extraction. Water samples subjected to physico-chemical analysis were filtered through GF/F glass fiber (0.7 μm, 47 mm diameter, Whatman, Tokyo, Japan) and immediately frozen until analyzed.

Physicochemical Parameter Analyses

Seawater sample analysis for nutrients and dissolved oxygen (DO/$mg\cdot L^{-1}$) were taken using 5-L GOFLO bottles at each layer according to the protocols of "The specialties for oceanography survey" [20, 21]. DO was determined using Winkler titrations [22]. Water samples were analyzed for nitrate (NO_3-N/$\mu mol\cdot L^{-1}$) and silicate (SiO_4-Si/$\mu mol\cdot L^{-1}$) with a SKALAR auto-analyzer (Skalar Analytical B.V. SanPlus, Holand). Ammonium (NH_4-N/$\mu mol\cdot L^{-1}$) and phosphorus (PO_4-P/$\mu mol\cdot L^{-1}$) were analyzed with methods of oxidized by hypobromite and molybdophosphoric blue. Dissolved oxygen (DO/$mg\cdot L^{-1}$) was determined using Winkler titrations. Total nitrogen (TN) and total phosphorus (TP) was measured according to Ameel et al. [22].

DNA Extraction, PCR Amplification and DGGE

The filters were cut into small pieces and transferred into extraction tubes, and then vortexed the tube briefly in 750 μl of TE. The community DNA extraction was performed modified according to Kozdroj and Elsas [23]. DNA concentration was measured spectrophotometrically (HiTaChi) and adjusted to a concentration of 100 $ng\cdot\mu l^{-1}$ for PCR purposes. A fragment of 16S rDNA (positon 341-907) was amplified by PCR using the primer sets 341F (5'-CCTACGGGAGGCAGCAG-3') and 907R (5'-CCGTCAATTC(A/C) TTT(A/G)AGTT-3'). 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') [10, 11]. The PCR was performed in a 50 μL reaction system containing 200 ng template DNA (negative controls with water), 1×Ex TaqTM Buffer, 200 μM dNTP, 0.4 μM of each primer, and 4U Ex polymerase (Takara Shuzo Co, Ltd, Otsu, Japan). Thermal cycling consisted of an initial denaturation step of 95ºC for 2 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s, and extension at 72ºC for 30 s, and a final elongation step at 72ºC for 30 min. The final elongation step was performed at 72ºC for 30 min in order to prevent the formation of artificial double bands in subsequent DGGE analysis [24].

Each PCR reaction was carried out in triplicate to reduce possible inter-sample PCR variations, and then pooled them together and purified before loading on the DGGE gel. PCR products were resolved by DGGE with an INGENY phorU-2 (Ingeny International BV, Netherlands) DGGE system. Equal amounts of PCR products (42 μL PCR product with 7 μL loading dye) of different layers were loaded on an acrylamide gel (1mm thick, 6% acrylamide) with a 45-70% linear gradient of denaturant (100% denaturant consisting of 7 M urea and 40% (v/v) for-

Table 1. Physicochemical parameters of different depths of northern SCS waters.

Code	Depth	Temperature	Salinity	TN	TP	Silicate	Phosphate	Nitrate	Ammonium	DO	Band
	(m)	(°C)	(‰)	(mg/L)	(mg/L)	(μmol/L)	(μmol/L)	(μmol/L)	(μmol/L)	(mg/L)	
11	D-0	29.78	34.59	5.16	1.36	0.45	0.0086	1.50	0.05	6.51	32
10	D-50	22.05	34.28	8.55	0.67	3.32	0.3490	3.20	0.31	5.65	38
9	D-75	18.10	34.52	11.11	0.66	12.75	0.0385	13.62	0.02	4.40	40
8	D-100	15.86	34.54	4.43	2.37	21.81	0.0202	18.06	0.02	3.90	36
7	D-150	13.88	34.52	ND	1.96	23.13	0.0155	15.01	0.06	3.93	36
6	D-200	11.18	34.45	30.10	2.67	35.78	0.0200	18.68	0.03	3.61	35
5	D-400	9.65	34.43	ND	2.74	56.72	0.0830	21.49	0.03	3.38	34
4	D-500	8.44	34.43	29.42	2.84	40.30	0.1130	28.06	0.05	3.02	33
3	D-600	7.56	34.43	34.96	2.83	82.38	0.7060	23.10	0.07	2.93	32
2	D-700	6.67	34.45	31.45	3.25	54.26	0.0850	23.51	0.03	2.77	30
1	D-900	4.03	34.51	33.95	4.06	81.62	0.0080	22.54	0.02	2.79	28

Codes for the depths are also applicable in the Fig.1.
D-0 means samples from surface layer, D-50 means sample from the depth of 50 m and so on.
ND means not determined

mamide). DGGE were performed at 60ºC during 17h at a constant voltage of 100 V. After electrophoresis, DGGE gels were stained with ethidium bromide and visualized under UV light using an AlphaImager imaging system (Alpha Innotech Corp., San Leandro, CA, USA).

Sequencing and Phylogenetic Analysis of DGGE Profile

Distinct DNA bands of interest were excised from the gel and resuspended in 20 μL of TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) and left at 4ºC overnight to elute DNA. The supernatant after centrifugation (12,000 rpm, 5 min, 4ºC) 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. The sequences obtained were analyzed against sequences in the Ribosomal Database Project (RDP) for chimeras by using the Ribosomal Database Project's CHECK-CHIMERA program firstly, and then use the classifier tool [25] and GenBank sequences using the Basic Local Alignment Search Tool (BLAST) program [26]. Phylogenetic trees of 16S rDNA partial sequences were generated using the neighbor-joining algorithms in Mega IV software [27]. The evolutionary distances were computed using the neighbor-joining method [27] and are 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 [28].

Data Analysis

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. Bands with a relative intensity of less than 0.5% of the sum of all band intensities were discarded. 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 Canonical correspondence analysis using CANOCO 4.5 for Windows (Biometris, The Netherlands), which can relate the quantitative changes (including band mobility and intensity) in bacterial community to water quality directly in order to investigate the relationship between bacterial assemblage and the environmental factors. Seven environmental variables related with water physicochemical properties (TN, TP, Nitrate, Silicate, Ammonium, and DO) were included. The analysis was done according to Zhang et al. [29].

Results

Environmental Characteristics and DGGE Pattern Analysis of the Investigated Samples at Different Layers

The basic physicochemical parameters of the investigated samples were summarized in Table 1. For instance, the surface water (0 m) has the highest temperature and salinity, the concentration of DO. Besides, it also showed the lowest concentration of silicate, phosphate, nitrate, and ammonium. While the deepest layer (900 m) had the lowest temperature, salinity, and the concentration of DO and the highest con-

centration of TP and silicate. The temperate range was from 4.02ºC to 29.78ºC. The range of the bands per sample was from 28 to 40 (mean 33.45), indicating a highly diverse bacterial assemblage in Xisha Island (Table 1).

DGGE Profiles

A total of 168 detectable bands in 78 different positions were detected in the DGGE gel. The most predominant 27 bands were successfully excised and sequenced. The sequence analysis result was summarized in Table 2 and Figs. 1 and 2. Based on Table 2, it showed that the dominant bacteria in Xisha Station related to Phylum *Cyanobacterium* (Y1, Y5, Y9, Y11, Y13, Y15, Y21, Y24, Y25, and Y29), Phylum *Protebacteria* (α-: Y2, Y3, Y4, Y12, Y16, Y18, Y19, Y22, Y23, Y27, and Y28; β-: Y7; γ-: Y17), Phylum *Firmicutes* (Y6), Phylum *Actinobacteria* (Y14 and Y26), and *Deinococcus-Thermus* (Y8). Sequence analysis showed that most of the sequences obtained were uncultured organisms present in environmental samples from sources such as South China Sea water, the East China Sea, marine biofilm, and coral reef related habits, including black band-diseased (BBD) coral tissues, mucus secreted by a *Pacillopora meandrina* coral colony, and reef water. The percentage similarity of clones and its closest blast ranged from 96% to 100%, respectively. Two sequences, bands Y14 and Y8, showed their similarities to the closest relatives obtained from GenBank database with no more than 97%, indicating that they might represent two new species. *Cyanobacteria* were only found in the upper 200 m (Fig. 3), with the highest diversity from the layer of D-75, while with depth increasing the percentage of *Protebacteria* also increased. The survey results also showed that some phylotypes were only present in the upper layers while some could be detected only in deep layers, such as Y4, Y10, and Y14 only appearing above 200 m, while Y19 and Y2 almost could be found in below 200 m (Fig. 1).

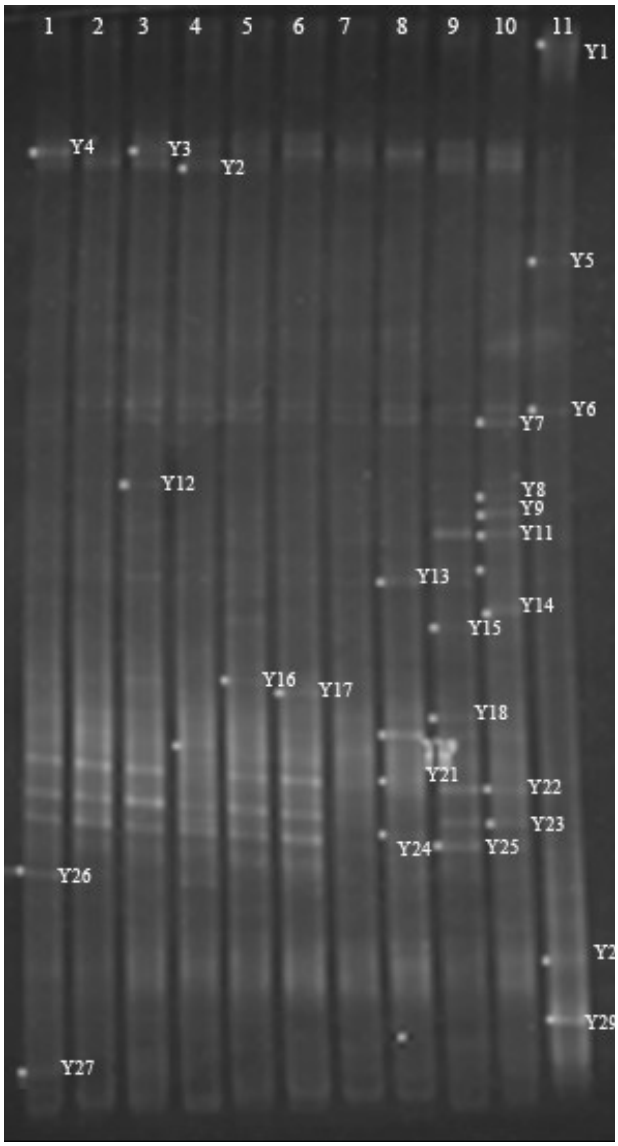

Fig. 1. The DGGE profile of vertical distribution of bacterial community in northern SCS and the sequences of the labeled DGGE bands that were excised and sequenced.

Cluster Anaylsis

UPMGA results showed that the samples tended to group together on the basis of depth. Fig. 4 indicated that all the samples can be divided into two groups: Group I: samples collected at 0 m, 50 m, 75 m, 100 m, 150 m; Group II: samples from 200 m, 400 m, 500 m, 600 m, 700 m, and 900 m. Group I can be further subdivided into two groups, bacterial assemblages obtained from depth 0 and 50 m are more similar, while samples from 75 m, 100 m, and 150 m are together in another subgroup. And Group II can further be divided into two subgroups. Analysis of DGGE fingerprints by UPMGA resulted in two clusters (Fig. 4) separated by the sampling depth. The first cluster contained layers no more than 150 m and the second contained the layer 200 m, 400 m, 500 m, 600 m, 700 m, and 900 m.

Relationship between Environmental Variables and Bacterial Community Structure

CCA was performed using all nine physicochemical parameters data as constrained variables of the bacterial community diversity obtained from DGGE fingerprinting of 0-900 m depth samples. CCA revealed values higher than 20 of the variance inflation factor for temperature, nitrate, silicate, and DO, which indicated that these variables were almost perfectly correlated with other variables in the dataset. Eigenvalues for the first two multivariate axes were 0.589 and 0.499, respectively. The sum of all canonical eigenvalues was 4.231. The CCA of the 16S rDNA DGGE data explained 13.9% and 11.8% of the variation in the first two axes (Fig. 5). And species environment correlations for both axes were 0.999, showing that bacteria were strongly correlated with environmental factors. Based on CCA, axis 1 was strongly correlated with TN and phosphorus, which showed positive correlation coefficient values of 0.5720 and 0.5747, respectively. Conversely, axis 2 had negative correlation with temperature and DO, with values of -0.6157 and -0.5038. Monte-Carlo significance

tests indicated only temperature (F=1.23, P=0.018, P<0.05) showing a significant correlation to the bacterial community structures.

Discussion

Microorganisms are critical components of marine ecosystems and play important roles in biogeochemical cycles, through affecting the rates of marine organic mineralization patterns. In order to understand the specific function, it is essential to investigate the bacterial community and its spatial variation in relation to the environmental factors [2, 3]. Based on Fig. 1, it showed that the dominant bacteria in Xisha area related to *Cyanobacteria* (Y1, Y5, Y9, Y11, Y13, Y15, Y21, Y24, Y25, and Y29), *Protebacteria* (α-: Y2, Y3, Y4, Y12, Y16, Y18, Y19, Y22, Y23, Y27, and Y28; β-: Y7; γ-: Y17), *Firmicutes* (Y6), *Actinobacteria* (Y14 and Y26) and *Deinococcus-Thermus* (Y8).

Cyanobacteria were only limited to the photic layer, with the detection of cyanobacterial phylotypes in the layer of 0 m, 50 m, 75 m, 100 m, 150 m, and 200 m. The comparison result of the cyanobacterial phylotypes investigated in this study and their closest blast relative in NCBI indicated that most of them were uncultured bacteria. *Synechococcus* was distributed abundantly in the layer 0 m, 50 m, and 100 m. Ma et al. [30] reported that the abundance of *Synechococcus* sp. and *Prochlorococcus* sp. in SCS were high, with higher abundance at surface water than at the depth of 80m and most of *Synechococcus* sp. being the MC-A group. During our investigation, we found that the *Synechococcus*-like cyanobacterium and the highest diversity of *Cyanobacteria* could only be detected in the upper 50 m layer. The sequence Y9 showed a 99% similarity with *Synechococcus* from the SCS mesoscale cyclonic eddy [35]. Diazotrophic *cyanobacteria* played an important role in marine primary production and marine nitrogen cycle by introducing new nitrogen to alleviate the nitrogen limitation in the oligotrophic area.

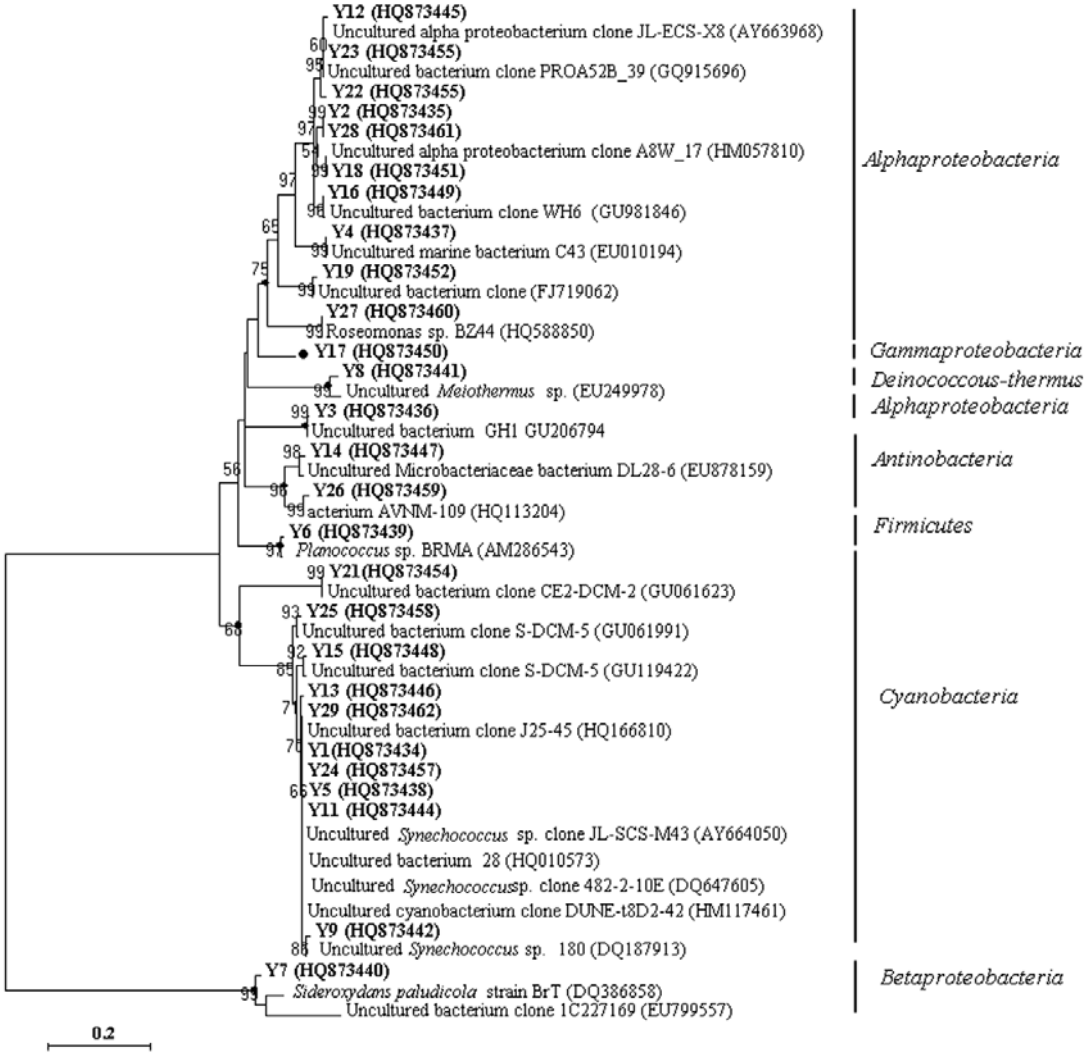

Fig. 2. Unrooted phylogenetic trees based on partial 16S rDNA sequences representing the respective DGGE bands in Fig. 3. Bootstrap analysis are based on 1,000 replicates. Bootstrap values from distance analysis are depicted. Bootstrap values less than 50% were not shown. Scale indicates 5% sequence divergence.

Table 2. Closest matches to excised and sequenced 16S rDNA-derived DGGE bands from bacterial communities of different depths.

Phylogenetic group	Sub-phylum	Band No.	Database match with accession number in parentheses	Origin	Identity %	Accession No.
Cyanobacteria		Y1	Uncultured bacterium DGGE gel band 28 (HQ010573)	Estuary sediment	100	HQ873434
		Y5	Uncultured *cyanobacterium* (HM117461)	Surface water in the Elbo Bay in the NW Meditrranean Sea	99	HQ873438
		Y9	Uncultured *Synechococcus* sp. (DQ187913)	100 meter deep seawater	99	HQ873442
		Y11	Uncultured bacterium (GU062129)	South China Sea	99	HQ873444
		Y13	Uncultured bacterium clone J25-45 (HQ166810)	Surface water	99	HQ873446
		Y15	Uncultured bacterium clone Reef_K20 (GU119422)	Reef water	99	HQ873448
		Y21	Uncultured bacterium CE2-DCM-2 (GU061623)	South China Sea	99	HQ873454
		Y24	Uncultured *Synechococcus* sp. clone JL-SCS-M43 (GU061991)	South China Sea	99	HQ873457
		Y25	Uncultured bacterium clone S-DCM-5 (GU061991)	South China Sea	98	HQ873458
		Y29	Uncultured *Synechococcus* sp. (DQ647605)	Marine biofilm	100	HQ873462
Proteobacteria	*Alpha-*	Y2	Uncultured alpha *proteobacterium* (EF123426)	Black band diseased (BBD) coral tissure	98	HQ873435
		Y3	Uncultured bacterium (GU206794)	South China Sea water	99	HQ873436
		Y4	Uncultured marine bacterium clone C43 (EU010194)	Seawater	99	HQ873437
		Y12	himia marina strain UDC301 (GQ245890)	Seawater	99	HQ873445
		Y16	Uncultured bacterium (GU981846)	Seawater	99	HQ873449
		Y18	Uncultured alpha *proteobacterium* (HM057810)	Ocean water from the Yellow Sea	98	HQ873451
		Y19	Uncultured bacterium (FJ719062)	Waquoit Bay National Estuarine Research	99	HQ873452
		Y22	Uncultured bacterium clone PROA52B_39 (GQ915696)	Within and outside of a Karenia brevis bloom	99	HQ873455
		Y23	Uncultured alpha *proteobacterium* clone JL-ECS-X8 (AY663968)	East China Sea	100	HQ873455
		Y27	*Roseomonas* sp. BZ44 (HQ588850)	Soil sample from an industrial site containing high amounts of heavy oil and heavy metals	99	HQ873460
		Y28	Uncultured alpha *proteobacterium* F1_22 (EF123426)	Black band diseased (BBD) coral tissues	100	HQ873461
	Beta-	Y7	Uncultured bacterium clone S25_529 (EF574185)	Site S25 near Coco's Island	99	HQ873440
	Gamma-	Y17	Uncultured bacterium (EU799557)	Newport Harbour, RI	98	HQ873450
Firmicutes		Y6	*Planococcus* sp. BRMA (AM2865430)	Phycosphere of *Chlorella vulgaris*	99	HQ873439
Actionbacteria		Y14	Uncultured *Microbacteriaceae* (EU878159)	Baltic Sea mesocosm experiment	97	HQ873447
		Y26	Bacterium AVNM-109(HQ113204)	Heavy industrial area	99	HQ873459
Deinococcous-thermus		Y8	Uncultured *Meiothermus* sp. (EU249978)	Mucus secreted by a *Pacillopora meandrina* coral colony	96	HQ873441

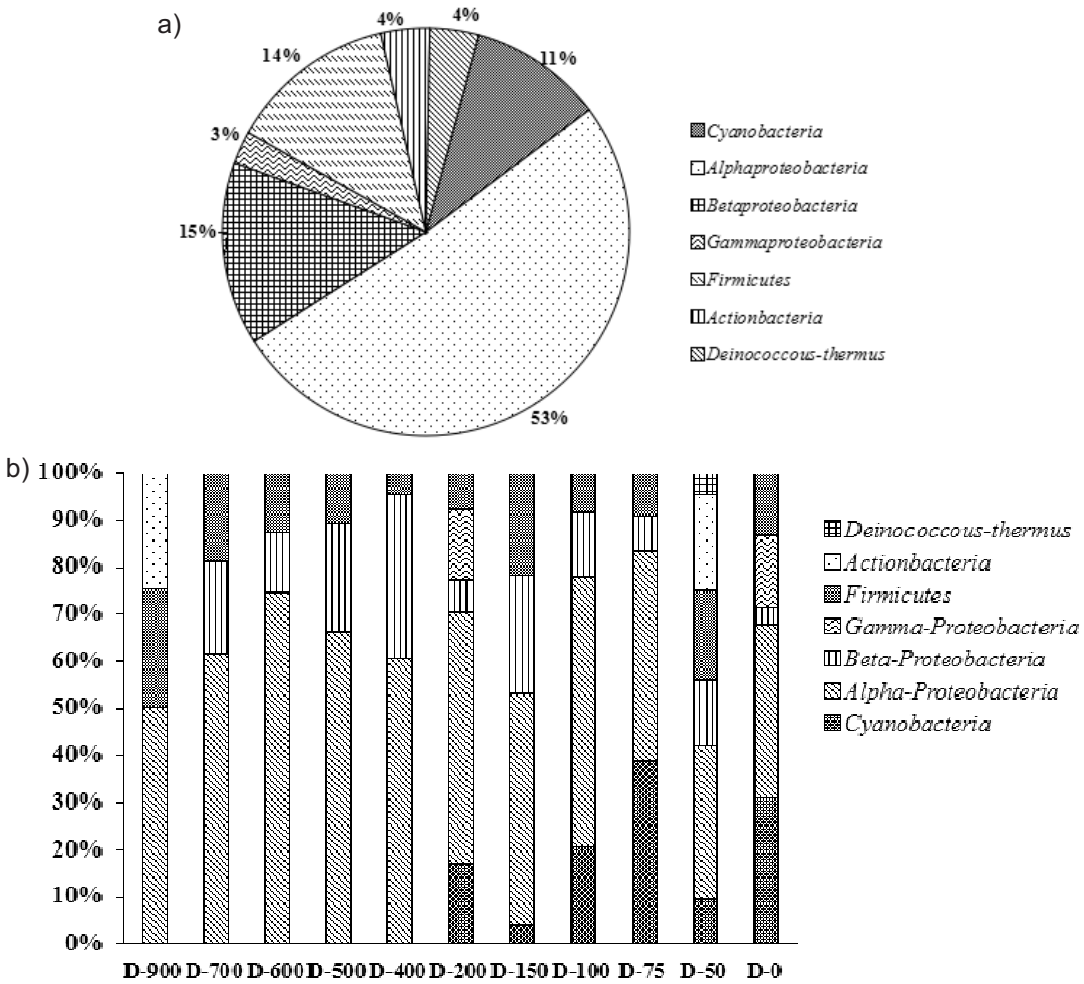

Fig. 3. Community structure comparison based on DGGE patterns of Fig. 1: a) analyses of the total detected bacterial species, b) bacterial community composition of different depths and the percentage of different phyla.

Protebacteria were the dominant bacteria, accounting for 51.2% of all the intense bands obtained from the DGGE profile of all the investigated layers, including *α-protebacteria*, *β-protebacteria*, and *γ-protebacteria*. The similarity range of the obtained sequences with existing Genbank records were from 94 to 100%, respectively. Sequence Y27 showed the highest similarity with *Roseomonas* species, which belongs to the genus *Roseomons* obtained from highly oil- and heavy metal-polluted areas (Table 2). *Roseomonas* sp. is an infectious bacterium related with human sepsis and other human infection diseases. It is also reported that *Roseomonas* sp. is capable of inhibiting the activity of acetylcholinesterase so that it can be applied in treating early senile dementia [32]. This finding indicated that the SCS harbored vast microbial resources with great potential for medical applications.

Based on Table 2, sequences Y2 and Y28 belonging to *α-protebacteria* showed the highest similarity with the bacteria associated with black band disease (BBD) coral tissue. They also have been found in other diseased corals so that they are assumed to be pathogenic bacteria to coral tissue. Research on bacterial communities associated with the coral reef in the Xisha area indicated that predominant bacteria were *α-protebacteria* [33]. BLAST results implied that sequence Y8 shared 96% identity with uncultured *Meiothermus* sp. (EU249978) from genus *Deinococcous-thermus* with the source of mucus secreted by a *Pacillopora meandrina* coral colony. As we all know that the Xisha area owns a long stretch of continuous coral reefs, it can be inferred that the obtained sequence Y8 in the investigated area may originate from the microbial community of coral reef and diffused into ocean due to seawater mixing or

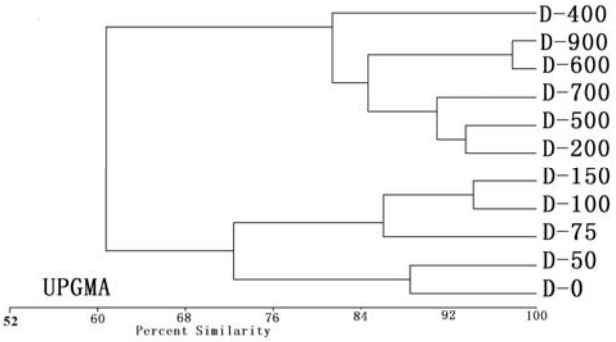

Fig. 4. Cluster analysis of DGGE gel pattern showing the amplified 16S rDNA gene fragments from different depths of bacterial community in northern SCS (samples indicated using sampling station codes in Table 1).

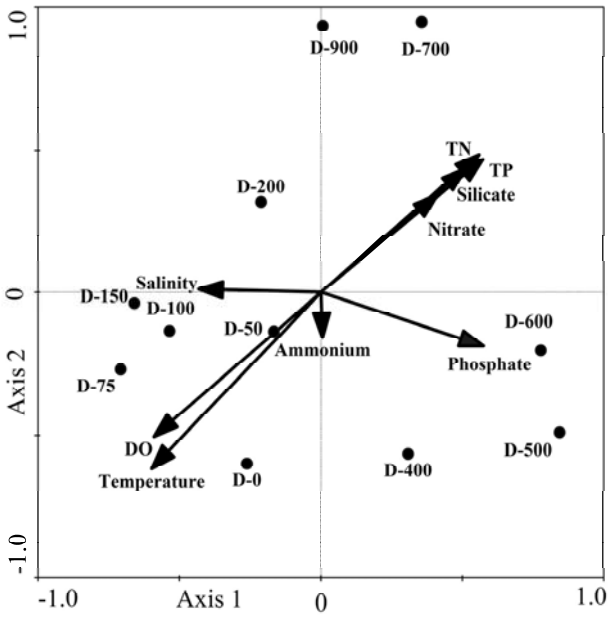

Fig. 5. CCA ordination diagram of DGGE data (dots represent samples and the sampling stations codes have been referred to in Table 1) with environmental factors (represented by arrows).

something else. In addition, *Deinococcous-thermus* possesses great radiation-resistant ability and can even eat nuclear pollution and some toxic substances. Due to this it can survive in very harsh and extreme environments. Therefore this microorganism could be used in the bioremediation process.

Vertical distribution of bacterial communities in the ocean related to many physicochemical factors, such as temperature, concentration of organic matter, and ocean circulation, so on [31, 34]. Ghiglione et al. [34] found that the vertical distribution (0-1000 m) of the bacterial community in the western Mediterranean consisted of three distinct subgroups: upper layer (0-40 m), shallow layer (60-150 m), and marine middle layer (200-1000 m). Concentration of organic matter plays a crucial role in distribution of the bacterial phylotypes. The cluster analysis result in this study was presented in 11 layers of the bacterial community that could be divided into 2 main groups: I, 0-150 m and II, 200-900 m. It can also be interpreted that all the bacteria consists of the upper photic zone section, the lower photic zone, and the nonphotic zone. The layer 150 m was a remarkable boundary for vertical distribution of bacteria. As in many parts of the open ocean ecosystem, the maximum amount of chlorophyll concentration values detected were found near the depth of 150 m, being the consequences of interaction among temperature, sunlight, and available nutrients, (e.g., inorganic and organic matter). Rooney-Varga et al. [35] and Sapp et al. [36] found that not only the abiotic factors but also biotic factors, such as the phytoplankton community, had an important impact on bacterial distribution; hence, all the factors related to the phytoplankton community pattern could work in limiting bacterial distribution. With the aim of elucidating the significant factors controlling the distribution of the marine bacteria, multivariate statistical methods such as CCA have been employed to analyze the relationship between the bacterial community structure and environmental factors, which have been applied widely on the research of the marine microbial community [5, 6, 36, 37]. CCA results suggest that temperature ($P=0.018$, $P<0.05$) was a significant factor together with other environmental factors in regulating the vertical distribution of bacteria here, which was consistent with results from Gao et al. [39] and Yan et al. [40]. The bacterial community responded differently to the concentration of phosphate, nitrate, and salinity at different depths, with the temperature, oxygen, dissolved organic carbon and solar radiation also affecting the bacterial community composition. The significant factor for the vertical and horizontal distribution of bacterial community is different, for the horizontal distribution the determining factor is nitrate concentration ($F=1.34$, $P=0.034$) while the investigation of vertical distribution in the Xisha area showed that temperature is the significant factor [6].

This work was intended to apply molecular techniques and statistical analysis to elucidate the vertical variations of the bacterial communities in relation to environmental factors of SCS water. However, this investigation just reflected the phylogenetic diversity of bacterial communities, therefore future work will study the abundance and the function of microbes coupled with environmental factors.

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