

Diversity and abundance of bacteria in the surface seawater of the Changjiang Estuary and its adjacent areas

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ABSTRACT

Question: What is the diversity and abundance of bacteria in the surface seawater of Changjiang Estuary and its adjacent areas?

Methods: We sequenced 1038 clones from surface seawater at ten different sites of Changjiang Estuary and its adjacent areas. We used culture-independent molecular approaches based on 16S rRNA genes to investigate diversity and abundance. We also used redundancy analysis to determine the relationship between bacterial diversity in surface seawater and various environmental parameters.

Results: Correlation analysis between environmental parameters and bacterial biomass indicated that dissolved oxygen and pH were statistically significantly correlated with bacterial biomass. Phylogenetic analysis of clone libraries indicated that Proteobacteria was the dominant taxon in all samples, followed by Bacteroidetes, Actinobacteria, Cyanobacteria, Firmicutes, Chloroflexi, Acidobacteria, Planctomycetes, Fibrobacteres, Verrucomicrobia, and Tenericutes. α -Proteobacteria was the dominant taxon within Proteobacteria. Cluster analysis and multidimensional scaling analysis revealed that bacterial communities could be classified into three groups (A02, A05/B03, and A01/A04/C04/A03/B02/C03/C05). Redundancy analysis indicated there were significant positive correlations between the distribution of bacterial community structure and pH, total suspended solids, total phosphorus, and dissolved oxygen.

Keywords: bacterioplankton, biomass, Changjiang Estuary, diversity, environmental variables, redundancy analysis.

INTRODUCTION

Bacteria play an important role in the marine ecosystem (Bacelar-Nicolau *et al.*, 2003) and their activity has a major impact on ecosystem metabolism and function (Gasol and Duarte, 2000), as well as the biogeochemical cycle (Tortell *et al.*, 1999). As the main primary producers,

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single-celled organisms, particularly bacteria, not only influence climate and dominate the transfer of nutrients and energy in marine environments, but also provide medicine and food for humans, and even play an important part in the origins of global environmental change (Kasting and Siefert, 2002). Prokaryotes, which are not usually considered by laypeople to be important in the marine food chain, are the basis of all interactions in marine environments (Karl, 2002). In aquatic ecosystems, microorganisms are very sensitive to changes in environmental conditions, and thus bacterial community composition can act as an environmental indicator (Paerl *et al.*, 2003). Previous studies have focused on the relationship between bacterioplankton and environmental factors (Donner *et al.*, 1996; Lindström, 2001; Liu *et al.*, 2009b), as well as the spatial distribution of bacterial communities (Sala *et al.*, 2008; Vieira *et al.*, 2008).

The Changjiang Estuary, known historically as the Yangtze River, is the third longest in the world. Because diluted Changjiang water mixes with Yellow Sea coastal water from the north and the Taiwan warm current from the south (Ning *et al.*, 2011), the Changjiang Estuary is extremely complicated and dynamic (Zhang *et al.*, 1999; Jiao *et al.*, 2007). Documenting the diversity and distribution of the bacterial communities in the Changjiang Estuary is a long-standing challenge, and little is known of bacterioplankton diversity and its influence on the environment. Liu (2009a) reported a relationship between bacterial abundance and concentrations of phosphates, nitrates, and chlorophyll *a*, involving dilution by the Changjiang River, upwelling, and suspended matter.

The objectives of this study were threefold. First, we wished to estimate the bacterial biomass of surface seawater of the Changjiang Estuary and its adjacent areas, based on DAPI fluorescence direct counts; here, we describe the correlation between environmental parameters and bacterial biomass. Second, based on clone libraries of 16S rRNA genes, we characterize the major prokaryotic populations and diversity in the estuary and adjacent areas. Third, using the VEGAN package in R, we perform cluster analyses to assess the correlation between bacterial community structure and environmental parameters.

MATERIALS AND METHODS

Sample collection

In July 2014, we collected samples at a depth of 2 m in surface seawater at ten sites along the Changjiang Estuary and adjacent areas, using an SBE 32 sampler (Sea-Bird Electronics, Bellevue, WA) (Fig. 1). Table 1 shows the latitude and longitude of the ten sampling sites. We collected samples for DAPI fluorescence analysis in 10-mL sterile cryopreservation tubes, and preserved them with buffered glutaraldehyde (GTA, final concentration of 1%), before storage in the dark at ambient temperature for 15 minutes. Subsequently, we stored the samples in airtight plastic bottles at -20°C for the duration of our time at sea, and at -80°C upon our return to the laboratory. We collected our samples for DNA isolation processing using a vacuum pump suction filter, at a vacuum of 20 kPa (Porter and Feig, 1980). We filtered samples of 1000 mL first through 3- μm , then 0.22- μm pore size polycarbonate nucleopore membranes (Merck Millipore Ltd., Billerica, MA). We preserved the 0.22- μm filters in 5 mL sterile cryopreservation tubes at -20°C while at sea and at -80°C after returning to the laboratory.

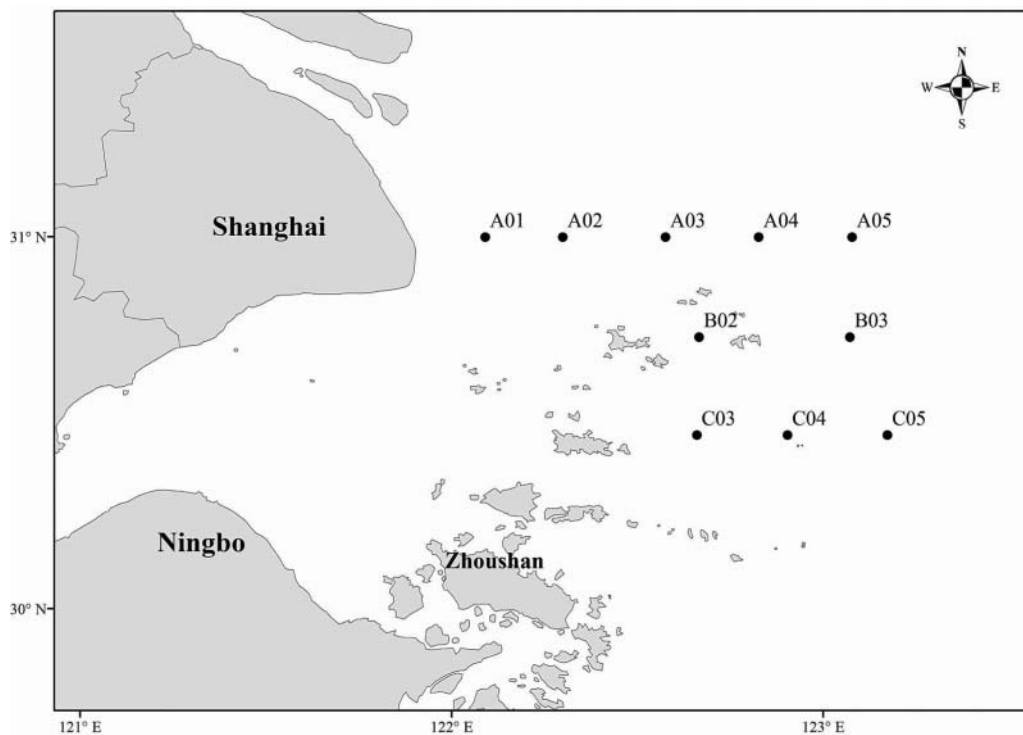


Fig. 1. Distribution of the ten sampling sites in the Changjiang Estuary. Full details of latitude and longitude are given in Table 1.

Environmental parameters

We pretreated samples following specifications for marine monitoring (National Standards of Peoples Republic of China, GB 17378.5, 2007) before chemical parameter analysis (Heijs *et al.*, 2008). We measured total nitrogen (TN) and total phosphorus (TP) content using a total organic carbon (TOC) analyser (Shimadzu Corporation, Kyoto, Japan). We measured total suspended solids (TSS) using gravimetric methods (Sartorius AG, Göttingen, Germany). We measured dissolved oxygen using a spectrophotometer (752 ultraviolet and visible spectrophotometer, Shanghai-Hengping, China).

DAPI fluorescence direct counts

We stained seawater samples with DAPI by adding 1 mL of pretreated sample to 1 mL of $20 \mu\text{g} \cdot \text{mL}^{-1}$ DAPI. Staining was performed in a darkened room over 30 minutes with occasional swirling of the reaction tube. We then poured the mixed solution through pre-wetted black polycarbonate nucleopore membrane filters (pore size $0.2 \mu\text{m}$) using a 5-mL syringe. We observed the membrane under a fluorescence microscope using a flat-field 100 $\times$ oil immersion lens, and counted at least 30 cells per filter falling within a minimum of 20 fields of view.

We calculated bacterial density in the original sample using the formula:

$$(\text{cells} \cdot \text{mL}^{-1}) = (N \times A_t)(A_g \times V_f)^{-1},$$

where N = the number of cells counted, A_t = the effective area of the filter (mm^2 or μm^2), A_g = the area of the counting grid (mm^2 or μm^2), and V_f = the volume of diluted sample filtered (mL) (Kepner and Pratt, 1994).

Correlation analysis

We used SPSS v.19 software (IBM) to determine correlations between environmental parameters and bacterial abundance.

DNA extraction and PCR

We processed samples according to the manufacturer's instructions using the FastPrep-24™ rapid nucleic acid extraction kit (MP Biomedicals, Santa Ana, CA). We measured the concentration of purified DNA using a protein nucleic acid detector (Bio-Rad, Hercules, CA). We amplified phylogenetically diagnostic sequences using the bacterial 16S rRNA universal primers 341f (5'-CCTACGGGAGGCAGCAG-3') and 907r (5'-CCGTC AATT CCTTTGAGTTT-3'), with PCR cycling as follows: 95°C for 5 minutes, then 35 cycles of 94°C for 1 minute, 56°C for 30 seconds, 72°C for 2 minutes, and a final elongation step of 72°C for 10 minutes. The samples were held at 4°C thereafter. The PCR mixtures (50 μL) contained 1 μL template DNA, 0.5 μL of each dNTP (10 $\text{nmol} \cdot \text{mL}^{-1}$), 1 μL (10 $\mu\text{mol} \cdot \text{mL}^{-1}$) of each primer, 0.5 μL (2.5 U) of Taq DNA polymerase, 41 μL ddH_2O , and 5 μL 10× PCR buffer (TaKaRa, Japan). The resulting PCR products were run on a 1.2% agarose gel in TAE buffer and visualized by staining with SYBR Gold under ultraviolet light. Bands of the expected size (approximately 550–700 bp) were cut out and purified using the MiniBEST Agarose Gel DNA Extraction Kit (version 4.0, TaKaRa, Japan) following the manufacturer's instructions, and then quantified using a NanoDrop ND-3000 (Thermo Scientific, Waltham, MA).

We cloned PCR products into the pMD18-T vector system (TaKaRa, Japan) using *Escherichia coli* DH5 α as the host. Positive clones were selected by blue-white selection. Libraries were screened for the 750-kb 16S rRNA insert by PCR with M13 primers. We obtained 16S rRNA gene sequences using an ABI 3730XL Automated Sequencer (Applied Biosystems, Waltham, MA).

Sequencing and phylogenetic analysis

We used Usearch to detect chimeric sequences, which were omitted from subsequent analysis. BLASTN (www.ncbi.nlm.nih.gov/BLAST/) was used to identify similar sequences as a backbone for phylogenetic analysis of the cloned sequences obtained here. Representative sequences of dominant genera were selected and aligned using the Cluster X program (Thompson *et al.*, 1994).

We calculated diversity (Shannon-Wiener index) and richness (Chou's abundance-based coverage estimator) for all samples on the basis of phylotype distribution (97% similarity), before clustering them as operational taxonomic units (OTUs) at an overlap identity cutoff

of 97% using DOTUR (Schloss and Handelsman, 2005). We considered sequences with >97% similarity to belong to the same phylotype. We calculated evolutionary distances of full-length sequences according to the Kimura two-parameter correction method (Kumar *et al.*, 2004), after which, using MEGA5, we constructed neighbour-joining trees with 1000 bootstrap resampling replicates.

Using the VEGAN package in R (Oksanen *et al.*, 2007), we employed multidimensional scaling (MDS) and cluster analysis to examine the correlations between sites. We employed redundancy analysis (RDA) to further examine the correlation between variation and environmental parameters. We applied the Hellinger transformation of species and sites data before performing the RDA (Legendre and Gallagher, 2001).

GenBank accession numbers

Nucleotide sequences for the 16S rRNA gene clones can be found under the following accession numbers: C04/KT731544–KT731563; A01/KT731564–KT731592; C05/KT731593–KT731624; B03/KT731625–KT731655; A05/KT731656–KT731686; A03/KT731687–KT731718; A02/KT731719–KT731781; A04/KT731782–KT731812; C03/KT731813–KT731862; B02/KT731863–KT731896.

RESULTS

Environmental parameters and bacterial counts

The physicochemical characteristics of seawater samples are given in Table 1. Briefly, site A02 had the highest total nitrogen (TN) and total phosphorus (TP), while site C05 had the lowest of each. Site B03 had the highest pH and site A03 the lowest; site B03 had the highest dissolved oxygen and site A02 the lowest; and site A01 had the highest total suspended solids (TSS) and site A05 the lowest. Table 1 shows DAPI fluorescence direct counts of 1 or 2 mL of seawater. Site B03 had the highest total bacterial counts and site A04 the lowest.

Correlation between environmental parameters and bacterial abundance

The correlations between environmental parameters and bacterial abundance are shown in Table 2. Two environmental factors – dissolved oxygen and pH (both $P < 0.05$) – were highly correlated with bacterial abundance.

Diversity

For each site, we screened approximately 110 clones for fragments of SSU rRNA of 550–700 bp. Sequences of more than 97% similarity were classified as single operational taxonomic units (OTUs). Clones, OTUs, and diversity indices of each clone library are shown in Table 3.

Table 1. Environmental parameters, total bacterial counts, and locations of the ten samples

Samples	TN (mg·L ⁻¹)	TP (μmol·L ⁻¹)	pH	DO (ml·L ⁻¹)	TSS (mg·L ⁻¹)	Total bacterial count (cells·mL ⁻¹)	Longitude	Latitude
A01	21.29 × 10 ⁻¹	2.51	7.93	4.15	387.40	1.61 × 10 ⁵	122°05'24.00"E	30°59'53.52"N
A02	22.31 × 10 ⁻¹	2.79	7.95	3.89	380.90	1.42 × 10 ⁵	122°17'57.48"E	30°59'53.52"N
A03	18.23 × 10 ⁻¹	1.61	7.92	4.31	44.00	1.55 × 10 ⁵	122°34'33.24"E	30°59'53.52"N
A04	16.86 × 10 ⁻¹	1.22	8.11	4.19	15.80	1.16 × 10 ⁵	122°49'33.24"E	30°59'53.52"N
A05	13.32 × 10 ⁻¹	0.90	8.30	5.58	14.30	1.23 × 10 ⁶	123°04'39.36"E	30°59'53.52"N
B02	14.90 × 10 ⁻¹	1.11	8.15	4.27	15.90	5.81 × 10 ⁵	122°39'58.68"E	30°43'45.84"N
B03	18.71 × 10 ⁻¹	0.50	8.39	5.90	22.40	1.48 × 10 ⁶	123°04'18.84"E	30°43'45.84"N
C03	20.29 × 10 ⁻¹	1.26	8.10	5.34	18.70	2.00 × 10 ⁵	122°39'36.00"E	30°27'56.52"N
C04	13.02 × 10 ⁻¹	0.30	8.33	5.49	15.10	3.49 × 10 ⁵	122°54'12.60"E	30°27'56.52"N
C05	2.38 × 10 ⁻¹	0.29	8.32	5.16	22.50	5.04 × 10 ⁵	123°10'22.80"E	30°27'56.52"N

Note: TN = total nitrogen, TP = total phosphorus, DO = dissolved oxygen, TSS = total suspended solids

Table 2. Correlation analysis between environmental parameters and bacterial abundance

		TN (mg·L ⁻¹)	TP (μmol·L ⁻¹)	pH	DO (ml·L ⁻¹)	TSS (mg·L ⁻¹)
Bacterial abundance (cells·mL ⁻¹)	Correlation coefficient, <i>R</i>	-0.213	-0.522	0.736	0.714	-0.318
	<i>P</i> (two-way, <i>P</i> < 0.05)	0.555	0.122	0.015*	0.020*	0.278
	<i>N</i>	10	10	10	10	10

Note: TN = total nitrogen, TP = total phosphorus, DO = dissolved oxygen, TSS = total suspended solids.
**P* < 0.05.

Table 3. Bacterial diversity indices

Sample	No. of clones	OTUs	Diversity indices			
			Bias-corrected Chao1	Abundance-base coverage estimator	Shannon-Wiener index (<i>H'</i>)	Simpson index (<i>D</i>)
A01	97	32	56.75	735.09×10^{-1}	2626.85×10^{-3}	1620.03×10^{-4}
A02	106	74	22.75×10	4603.34×10^{-1}	4060.21×10^{-3}	172.81×10^{-4}
A03	104	33	90.75	978.62×10^{-1}	2748.81×10^{-3}	1067.96×10^{-4}
A04	101	33	7.15×10	1141.25×10^{-1}	2663.79×10^{-3}	1324.75×10^{-4}
A05	106	31	0.44×10^2	486.23×10^{-1}	2951.82×10^{-3}	697.22×10^{-4}
B02	107	35	0.51×10^2	546.98×10^{-1}	3226.13×10^{-3}	437.87×10^{-4}
B03	95	35	57.67	561.21×10^{-1}	3251.04×10^{-3}	387.46×10^{-4}
C03	109	52	126.38	1290.25×10^{-1}	3535.89×10^{-3}	387.36×10^{-4}
C04	107	21	39.33	354.38×10^{-1}	2124.83×10^{-3}	1996.12×10^{-4}
C05	106	33	0.71×10^2	833.75×10^{-1}	2866.45×10^{-3}	819.41×10^{-4}

Note: OTUs = operational taxonomic units.

We used Chao1 and Chao's abundance-base coverage estimator (ACE) to estimate the number of species in each community. All indices showed the same trends. The number of species was highest in the A02 and C03 libraries, and lowest in the C04 library. The Shannon-Wiener and Simpson indices showed the same trend for species diversity. The rarefaction curves of each of the ten clone libraries (Fig. 2) show the same trend, with A02 and C03 still rising at the maximum number of clones, while other samples plateau.

Clone libraries of 16S rRNA genes

BLASTN was used to identify the sequences obtained or to find ones similar to those obtained here, and MEGA5 was used to construct a phylogenetic tree (data at evolutionary-ecology.com/data/Appendix3081.pdf). Unclassified or unidentified OTUs were classified from the ten samples, giving 1038 clones altogether, belonging to 11 known divisions (Fig. 3): Proteobacteria (61.37%), Bacteroidetes (15.14%), Actinobacteria (14.93%), Cyanobacteria (4.82%), Firmicutes (1.16%), Chloroflexi (0.77%), Acidobacteria (0.39%), Planctomycetes (0.19%), Fibrobacteres (0.19%), Verrucomicrobia (0.19%), Tenericutes (0.10%), and unclassified (0.48%). Proteobacteria included α-Proteobacteria (51.44%), β-Proteobacteria (4.24%), γ-Proteobacteria (4.14%), δ-Proteobacteria (1.45%), and

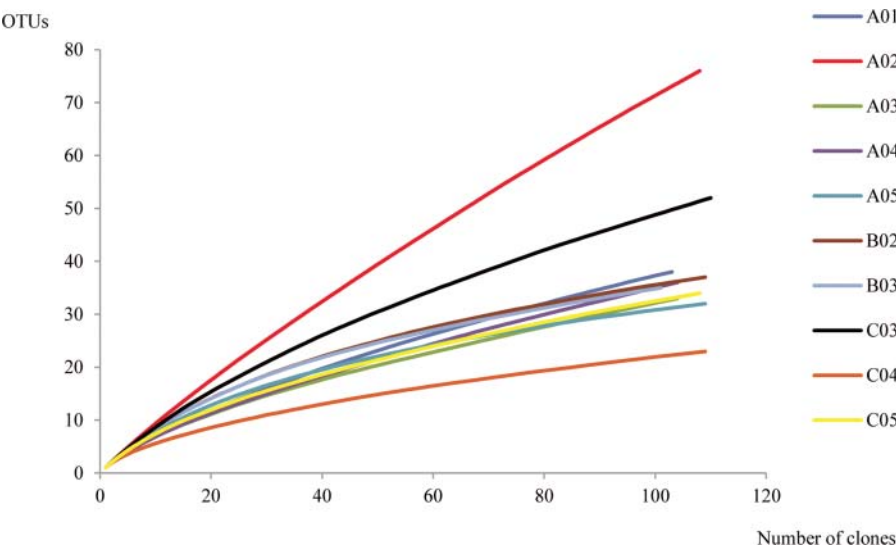


Fig. 2. Rarefaction curves of the ten clone libraries.

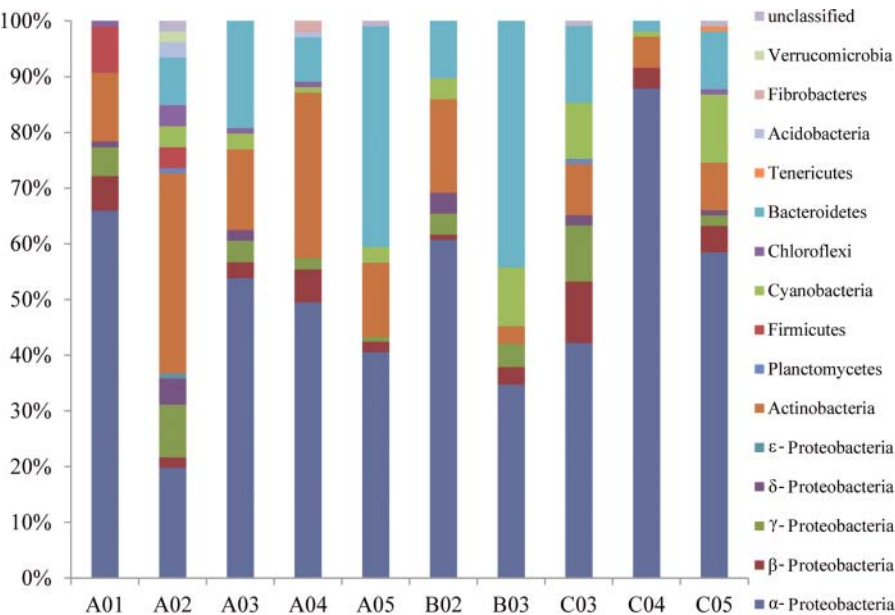


Fig. 3. Clone library ratios of each bacterial group, based on number of operational taxonomic units (OTUs) obtained.

ϵ -Proteobacteria (0.10%). Proteobacteria was the major component in each of the libraries except B03, with Proteobacteria, Bacteroidetes, and Actinobacteria dominating the libraries. α -Proteobacteria was the major component of Proteobacteria; the other Proteobacteria were less abundant, especially δ -Proteobacteria and ϵ -Proteobacteria in each library. ϵ -Proteobacteria were detected in the A02 library only, at a low abundance (0.94%), together with Verrucomicrobia (1.87%). Tenericutes were found in the C05 library only (0.94%) and Fibrobacteres in the A04 library (1.98%) only. Actinobacteria were present in all ten libraries.

Spatial distribution of bacterial communities

Results of multidimensional scaling (MDS) and cluster analysis are shown in Fig. 4. Based on the cluster analysis, bacterial communities can be classified into three groups based on relative abundances: A02, A05/B03, and A01/A04/C04/A03/B02/C03/C05. MDS based on the relative abundance of 16S rRNA gene sequences showed the same results.

To find potential correlations between the distribution of bacterial communities and the environmental conditions, we conducted a redundancy analysis (RDA) (Fig. 5). The RDA revealed the main environmental factors on axis-1 to be dissolved oxygen and pH (positive correlation in each case); total nitrogen (TN), total phosphorus (TP), and total suspended solids (TSS) were the main environmental factors on axis-2 (also positive correlations). Applying the envfit() function to each environmental factor (Table 4) resulted in very low Pr values of TP, pH, dissolved oxygen, and TSS, with significant positive correlations between the distribution of bacterial communities and TP, dissolved oxygen, pH, and TSS.

DISCUSSION

Bacterial abundance and its correlation with environmental factors

DAPI fluorescence direct enumeration showed that sites A05 and B03 had the highest total bacterial counts, while A04 had the lowest, with an average of 4.92×10^5 cells $\cdot$ mL $^{-1}$. This result is in line with previous reports. Zhao *et al.* (2003) reported abundances ranging from 3.05×10^5 cells $\cdot$ mL $^{-1}$ to 1.36×10^6 cells $\cdot$ mL $^{-1}$, with an average of 6.09×10^5 cells $\cdot$ mL $^{-1}$ in

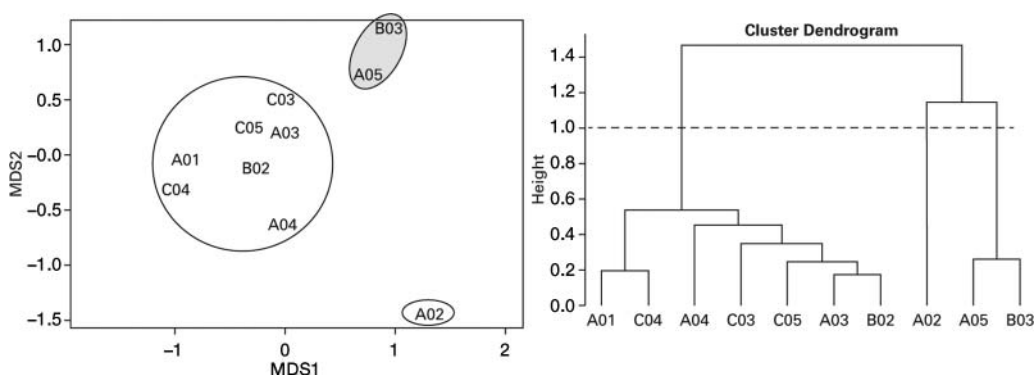


Fig. 4. Multidimensional scaling (MDS) and cluster dendrogram showing similarity of OUT composition among the ten sites in the Changjiang Estuary and adjacent areas.

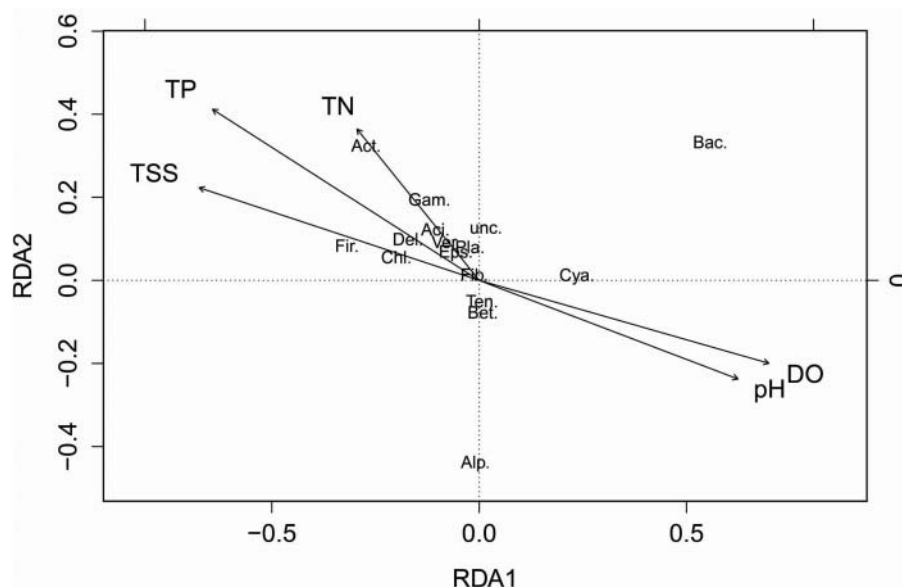


Fig. 5. Ordination diagram of redundancy analysis (RDA) of bacteria. Environmental variables: TP = total phosphorus, TN = total nitrogen, TSS = total suspended solids, DO = dissolved oxygen, plus pH. Species: Alp, α -Proteobacteria; Bet, β -Proteobacteria; Gam, γ -Proteobacteria; Del, δ -Proteobacteria; Eps, ϵ -Proteobacteria; Bac, Bacteroidetes; Act, Actinobacteria; Cya, Cyanobacteria; Fir, Firmicutes; Chl, Chloroflexi; Aci, Acidobacteria; Pla, Planctomycetes; Fib, Fibrobacteres; Ver, Verrucomicrobia; Ten, Tenericutes; unc, unclassified.

Table 4. Correlation analysis of environmental factors and bacterial community structure in the Changjiang Estuary and adjacent areas

Environmental variable	RDA1	RDA2	r^2	Pr
Total nitrogen	-70.829×10^{-2}	70.592×10^{-2}	3.010×10^{-1}	0.278
Total phosphorus	-85.533×10^{-2}	51.808×10^{-2}	8.352×10^{-1}	0.003**
pH	92.266×10^{-2}	-38.561×10^{-2}	6.378×10^{-1}	0.018*
Dissolved oxygen	94.484×10^{-2}	-32.753×10^{-2}	7.490×10^{-1}	0.003**
Total suspended solids	-93.462×10^{-2}	35.564×10^{-2}	7.208×10^{-1}	0.021*

* $P < 0.05$, ** $P < 0.01$. Number of permutations: 999.

the East China Sea. These values are two orders of magnitude higher than those reported by Lin (1998) for the Pacific Ocean and Prydz Bay in Antarctica, possibly as a result of the differences in pollution levels between the locations. The Changjiang Estuary is heavily polluted by industrial, agricultural, and domestic wastewater, all of which provide a rich nutrient supply for bacterial growth.

Two environmental factors – dissolved oxygen and pH – were highly correlated with total bacterial abundances, indicating that dissolved oxygen and pH are the main factors governing the bacterial distribution in our samples. Similarly, Li *et al.* (2005) found that

bacterial abundance was closely correlated with dissolved oxygen in Bohai Bay (Hebei Province, China), while Bacelar-Nicolau *et al.* (2003) found that bacterial abundance was closely correlated with pH in the Mondego Estuary (Portugal). The nutrients N and P are necessary for the growth of bacteria, and many studies in both freshwater and marine environments have shown that inorganic nutrients may be the limiting factors for the growth of bacteria (Yannarell *et al.*, 2003; Zeng *et al.*, 2009). There was no correlation between bacterial abundance and TP and TN, indicating that the nutrients were sufficiently saturated to support the bacterial populations.

Diversity of bacteria

Analysis of the 16S rRNA gene libraries revealed that Proteobacteria, Bacteroidetes, and Actinobacteria were the dominant taxa present. Feng *et al.* (2009) studied the bacterial diversity of water and sediment in the Changjiang Estuary, showing that Proteobacteria were predominant in the bacterial community. Proteobacteria accounted for 72.9% of all clones, of which α -Proteobacteria and γ -Proteobacteria were the dominant taxa. Sekiguchi *et al.* (2002) studied the bacterial distribution and phylogenetic diversity of the Changjiang Estuary, and also found α -Proteobacteria and γ -Proteobacteria to be the predominant taxa. Kan *et al.* (2006) found α -Proteobacteria to be predominant in Chesapeake Bay. All of these studies show α -Proteobacteria to be abundant in diverse systems, making up more than 50% of the bacterial biomass. The present study shows that Proteobacteria account for 61.37% of all clones, being the dominant component in the Changjiang Estuary and adjacent areas.

Many studies have reported the members of α -Proteobacteria to be highly salt-resistant and able to grow using a carbon resource such as dissolved organic carbon (Sjöstedt *et al.*, 2012). Five of the samples in the present study had more than 50% of their bacterial biomass identified as α -Proteobacteria, despite the strong gradient of salinity and nutrient contents from the Changjiang Estuary to the sea.

β -Proteobacteria (4.24%) and γ -Proteobacteria (4.14%) were the next most dominant taxa. β -Proteobacteria were present in every sample, in line with previous results showing β -Proteobacteria to be the dominant bacteria in the freshwater lakes and estuaries of the world (Cottrell *et al.*, 2005; Mueller-Spitz *et al.*, 2009). γ -Proteobacteria are widely distributed and dominant in marine sediments, but not in the water column (Bowman and McCuaig, 2003; Inagaki *et al.*, 2003).

δ -Proteobacteria (1.45%) and ϵ -Proteobacteria (0.10%) were also present, though at low levels. δ -Proteobacteria can use diverse electron donors or acceptors, including oxygen, sulfide, nitrate, and hydrogen. Musat *et al.* (2009) noted that δ -Proteobacteria are capable of breaking down alkanes, toluene, benzene, and hydrocarbons, all of which arise from the degradation of oil. Sample sites that include δ -Proteobacteria may be polluted by petroleum hydrocarbons. Nakagawa *et al.* (2005) suggest that ϵ -Proteobacteria mainly use nitrates in sediment as an electron acceptor; in the present study, they were found only at site A02, located near the Changjiang Estuary, which is rich in nutrients.

Bacteroidetes, Actinobacteria, and Cyanobacteria were distributed across our sample sites. Bacteroidetes mainly included Flavobacteria and Sphingobacteria. Flavobacteria are found in various aquatic environments and are related to algal red tides and degradation processes (Abell and Bowman, 2005). Sphingobacteria can degrade cellulose (Li *et al.*, 2011) and are more widely distributed in marine environments. This suggests that Actinobacteria in the Changjiang Estuary and its adjacent areas can be considered an important resource. They

are widely distributed, are abundant in marine environments, and are popularly believed to be an important source of bioactivators and potential drug compounds (Bull *et al.*, 2005). Cyanobacteria are widely distributed in marine environments and can perform oxygenic photosynthesis, which plays an important role in primary production and are important for the stability and diversity of marine ecosystems (Sun *et al.*, 2002).

We also detected Firmicutes, Chloroflexi, Acidobacteria, Planctomycetes, Fibrobacteres, Verrucomicrobia, each over a small range. Firmicutes were found mainly in samples A01 and A02; Vieira *et al.* (2008) reported that an abundance of Firmicutes suggests polluted water. Chloroflexi is the third bacterial phylum (after Proteobacteria and Nitrospirae) that can gain energy from nitrite oxidation (Sorokin *et al.*, 2012). Its members were found only in offshore samples with abundant nutrients. Barns *et al.* (2007) reported that Acidobacteria mostly exist in acidic conditions, which suggests acidification of the waters of the Changjiang Estuary and adjacent areas.

Members of Tenericutes and the SAR marine bacterioplankton clade were also observed. Relatively few reports exist of Tenericutes: Hou *et al.* (2011) reported them from hypersaline Qinghai lake sediment, and Beazley *et al.* (2012) identified them from the coastal salt marshes of the Gulf of Mexico affected by the *Deepwater Horizon* oil spill. Therefore, we speculate that the Tenericutes are adapted to conditions of high salinity and petroleum hydrocarbon pollution. SAR11 was the first clade of bacterioplankton reported to be widely distributed in subtropical waters; Rappé *et al.* (2002) suggested that SAR11 is involved in the regulation and control of the circulation of dissolved organic matter, playing an important role in carbon and nitrogen cycles. Previous research has reported the SAR406 clade to be present in anoxic environments, such as oxygen minimum zones in the ocean (Wright *et al.*, 2012). Both SAR11 and SAR406 were found in sample A02, where dissolved oxygen was lowest, confirming previous observations.

The relationship between community structure and the environment

Previous research has shown that bacteria respond quickly to changes in biotic and abiotic conditions, such as salinity, oxygen content, and particulate matter concentrations. Stratification of conditions in the marine water column may be the basis for a pronounced stratification of microbial populations (Giuliano *et al.*, 1999). In the present study, the results of the correlation analysis combined with those of the RDA indicate that total phosphorus, dissolved oxygen, pH, and total suspended solids (TSS) are the environmental factors with the most influence on bacterial community structure in surface seawater of the Changjiang Estuary and adjacent areas.

Previous research has reported that the growth of bacteria depends on suitable acid–base chemistry, and that different groups of bacteria have a different optimum pH range (Liu *et al.*, 2012). Yannarell and Triplett (2005) reported that bacterial community composition was significantly correlated with pH in 30 lakes in northern and southern Wisconsin, USA. Guo *et al.* (2012) found that the distribution of bacterioplankton in surface waters near Leizhou Peninsula (Guangdong Province, China) is significantly positively correlated with pH, which is consistent with our results. In Changjiang Estuary, pH ranged from 7.92 to 8.39, a weakly alkaline marine environment that permits the growth of most types of bacteria, with buffering from seawater permitting adaptations to changes in pH. The significant positive correlation between the distribution of bacterial community structure and pH may be due to the combined influence of other environmental factors.

Suspended solids are ubiquitous in natural water bodies. Samples in the present study were collected from areas of high turbidity and complex hydrological conditions. Organic nutrients are adsorbed at surfaces because many aquatic bacteria are adapted to grow on surfaces (Jannasch and Pritchard, 1972; Zobell, 1943). They are often found attached to suspended solids in substantial quantities both in freshwater and seawater environments (Seki, 1970; Paerl, 1975). In our study, TSS was significantly positively correlated with the distribution of bacterial communities, which was consistent with a previous study in the Oosterschelde Basin in the Netherlands (Laanbroek and Verplanke, 1986). Furthermore, Shi *et al.* (1992) found that TSS was a key factor in determining bacterial abundance and distribution in the Changjiang Estuary and adjacent areas.

Bacterioplankton are also affected by nutrients (Biddanda *et al.*, 2001). The Changjiang Estuary is badly polluted, mainly through phosphates and dissolved inorganic nitrogen (Liu *et al.*, 2009a), and phosphorus limitation of bacterioplankton growth has been demonstrated in several seas (Cotner *et al.*, 1997; Zweifel *et al.*, 1993). In the present study, total phosphorus was a limiting factor for the distribution of bacterial community structure, whereas total nitrogen was not, which is consistent with work by Jiang *et al.* (2014) in the northern South China Sea. The Changjiang Estuary has been eutrophic for decades, so nitrogen is unlikely to be a limiting factor there. Li and Li (2007) reported that the factors responsible for the regulation and restriction of bacterial growth and distribution differ among marine environments and among ecosystems generally. Many previous studies have reported oxygen minimum zones in the East China Sea (Zhang, 1990; Yang *et al.*, 2001). And Yan *et al.* (2008) reported that bacterial community structure responds quickly to changes in dissolved oxygen. In the present study, dissolved oxygen was the main factor governing bacterial abundance in areas with low dissolved oxygen.

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