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Dynamics of the bacterial and archaeal communities in the Northern South China Sea revealed by 454 pyrosequencing of the 16S rRNA gene

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ABSTRACT

Dynamics of the prokaryote (bacteria and archaea) abundance and community compositions in the Northern South China Sea (NSCS) in August 2009 and January 2010 were studied by flow cytometric analysis and 454 pyrosequencing of the 16S RNA gene. Prokaryotic community structures in the NSCS varied across space and over time, and this variation was strongly correlated with NO_3^- concentration. Prokaryote in estuarine and coastal waters was more abundant, but relatively less seasonally dynamic than in the open ocean. Major bacterial and archaeal lineages showed different niche preferences. Archaeal community was dominated by Marine Group I and Marine Group II. Clusters of Marine Group I varied spatially, while clusters of Marine Group II varied seasonally. *Synechococcus* and *Prochlorococcus* were two major autotrophic bacteria found in the NSCS. *Synechococcus* prevailed at the estuarine station in summer, while *Prochlorococcus* had high abundance at open-ocean stations in summer. Subcluster 5.2 *Synechococcus* and Sub 5.1 clade II *Synechococcus* were the dominant *Synechococcus* lineages in the NSCS, with the former dominating in the estuary during summer and the latter dominating at all other stations. Our results suggest that prokaryotic assemblages are highly complex in the NSCS and are controlled by seasonal monsoon and river discharge, showing spatiotemporal variations.

1. Introduction

Prokaryotes, which constitute the two domains bacteria and archaea, are the most abundant and diverse organisms in marine waters (DeLong, 1992; DeLong et al., 1994; Curtis et al., 2002; Pedrós-Alió, 2006; Alonso-Sáez et al., 2011). These highly diverse organisms are involved in the cycles of virtually all essential elements in marine environment such as carbon and nitrogen cycles. Their essential role in decomposing and recycling organic matter has been well recognized. Community composition, biomass and activity of these organisms critically influence carbon fluxes of marine ecosystems (Mou et al., 2008; Jiao et al., 2010a; Gantner et al., 2011). Autotrophic bacteria for example, contribute up to 60% of the total primary production in the open ocean ecosystem (Platt et al., 1983). Heterotrophic bacteria on the other hand, consume around 20–60% of total primary production in marine ecosystems (Fuhrman, 1992; Kirchman et al., 1993) and turn dissolved organic carbon into higher trophic levels via the microbial loop (Azam, 1998). Moreover, heterotrophic bacteria have recently been recognized as playing an important role in marine carbon reservation by the microbial carbon pump (Jiao et al., 2010a).

Comparing to bacteria, archaea are less diverse and not well-studied organisms. Archaea, previously found to distribute only in extreme environment, were later found widely distributed in marine waters with high abundance (DeLong et al., 1994; DeLong and Pace, 2001). It has been reported that two main archaeal phyla, Euryarchaeota and Crenarchaeota, occupy different niche in marine ecosystems (Massana et al., 1997; DeLong, 2003). Euryarchaeota are more abundant in surface waters, whereas Crenarchaeota are more abundant at depth (Massana et al., 1997; Alonso-Sáez et al., 2011). Study carried out at the Hawai'i Ocean Time-series station ALOHA in the North Pacific subtropical gyre showed pelagic Crenarchaeota represents one of the ocean's most abundant single cell types (Karner et al., 2001). In some regions, they account for more than 20% of the total picoplankton cell densities (DeLong, 2003).

Understanding the variations of microbial communities in the ocean helps us to understand the long-term responses of marine microbes to environmental changes (Giovannoni and Vergin, 2012). Based on analysis of the 16S rRNA gene and flow cytometry, seasonal and spatial variations of bacterioplankton communities have been studied in many coastal and oceanic waters, such as the western English Channel (Gilbert et al., 2009, 2011), the Southern Ocean (Jamieson et al., 2012), southern California (Dillon et al., 2009) and the Mediterranean Sea (Ruiz-González et al., 2011). Many of these studies revealed apparent seasonal and spatial

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dynamics of bacterioplankton communities, and some studies pointed out that certain bacterioplankton orders (such as SAR11 and *Rhodobacterales*) had different seasonal abundances (Gilbert et al., 2011). These studies identified a number of different environmental parameters, such as NH_4^+ and total organic nitrogen concentration (Gilbert et al., 2011), phosphate concentration (Gilbert et al., 2009), temperature (Gilbert et al., 2009; Jamieson et al., 2012) and salinity (Dillon et al., 2009; Fortunato et al., 2012), affecting the dynamics of bacterioplankton communities. However, there are few studies assessing the seasonal variation of archaeal community in marine environment (Murray et al., 1998; Herfort et al., 2007; Winter et al., 2009; Hollibaugh et al., 2013).

The South China Sea is a large marginal sea in the western tropical North Pacific Ocean. Its northern part is influenced by a variety of different oceanographic processes, including freshwater discharge from the Pearl River, mesoscale eddies, coastal upwelling and the interaction of different currents, modulated by the East Asian monsoon. The highly dynamic and complex hydrological condition affects the composition and function of bacteria communities along the nutrient gradient from river plume to the oligotrophic basin in different seasons. This in turn might explain the seasonal variation of carbon fluxes in this region. However, only few studies addressing bacterial and archaeal community compositions and their seasonal variations in the Northern South China Sea (NSCS) have been done so far.

In recent years, high-throughput sequencing, such as 454 pyrosequencing, has been applied in the research of bacterial community composition and diversity. These applications provide less biased, more robust and high-coverage results that help to uncover many details about the distribution of bacteria in a broad range of environments. In this study, we studied the seasonal variations of free living bacterial and archaeal community compositions along a transect from the estuarine water of the Pearl River to the oligotrophic basin in the NSCS by applying the 454 pyrosequencing method.

2. Methods

2.1. Sample collection, analyses of environmental parameters and prokaryote abundances

Samples were collected from surface at 5 stations along a transect from the Pearl River estuary to the oceanic region of the NSCS in August 2009 and January 2010 (Fig. 1, Table 1). Seawater samples were collected using Niskin bottles (12 L) attached to a conductivity, temperature, and depth (CTD) rosette multi-sampler. Environmental parameters (nitrate (NO_3^-), phosphate (PO_4^{3-}), dissolved oxygen, and chlorophyll *a* (Chl *a*) concentrations) were measured as described in Kong et al. (2011) (Table 1). Temperature was measured by the CTD. Picoplankton cell densities were quantified using flow cytometry (FCM) following the methods described in Chen et al. (2012) and Liu et al. (2014).

At each station, 1 L of seawater was filtered successively through a 3.0 μm and 0.22 μm (47 mm) polycarbonate membranes (PALL Corporation). Samples retained on 0.22 μm membranes were stored at −80 °C immediately after filtration, and used for DNA extraction.

2.2. DNA extraction, PCR and pyrosequencing

DNA was extracted using enzyme/phenol–chloroform protocol (Riemann et al., 2008). DNA was eluted in TE buffer (Tris–EDTA buffer) and kept at −20 °C until further analysis. The bacterial 16S rRNA gene was amplified using barcoded primers 341F (5′-adaptor+barcode+CCTAYGGGRBGCASCAG-3′) and 806R (5′-adaptor+GGACTACNNGGGTATCTAAT-3′) (Yu et al., 2005). Archaeal 16S rRNA gene was amplified using barcoded primers 340F (5′-adaptor+barcode+CCCTAYGGGGYGCASCAG-3′) and 1000R (5′-adaptor+GGCCATGCACYWCYTCTC-3′) (Gantner et al., 2011). For bacterial 16S rRNA gene, PCR reactions contained a final concentration of 0.5 mM $MgCl_2$, 0.5 μM of each primer (Yu et al., 2005), 0.8 mM of each dNTP and 1.0 unit of hot start polymerase (Invitrogen, USA). The template DNA concentration was about 10 ng per reaction (the template DNA was diluted to 10 ng μl^{-1}, and 1 μl was added). The PCR cycles started with a 5 min initial denaturation at 95 °C, followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s. A final extension of 72 °C for 5 min was included before holding at 4 °C. For the amplification of 16S rRNA gene of archaea, protocol of Stephan Gantner was followed (Gantner et al., 2011). All the reactions were done in 25 μl reaction volume in triplicates.

PCR products were gel-purified using the Qiaquick gel purification kit (Quiagen, Hilgen, Germany). Library quantification was done by fluorometry using the Quant-iT picoGreen dsDNA Assay Kit (Invitrogen, USA). An equal volume of each amplicon was mixed to prepare amplicon pools, and then sequenced in a two-region 454 run on a GS PicoTiterPlate using a Roche/454 GS Junior pyrosequencing system (Roche, 454 Life Sciences, Branford, CT, USA).

2.3. Data analysis

Analysis of 16S rRNA data was conducted using the microbial ecology community software program Mothur (http://www.mothur.org/wiki/Download_mothur) (Schloss et al., 2009). The reads were processed by removing tags and primers, only accepting reads with an average quality score above 20 and read lengths longer than 300 nt. Data analysis was carried out following the Schloss standard operating procedure (Schloss et al., 2009). Sample coverage, ACE richness estimators and Shannon diversity index were calculated at 97% similarity. A dendrogram describing the similarity of the samples was generated using Thetayc calculators. Sequences were classified using the greengene database (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) and NCBI Blast (http://www.ncbi.nlm.nih.gov/).

A neighbor-joining phylogenetic tree of Cyanobacteria 16S rRNA genes was constructed in MEGA 4 using representative sequences at a 0.03 genetic distance (Tamura et al., 2007). Nearest relatives were retrieved from the NCBI database. A heatmap showing the relative number of sequences per sample for each operational taxonomic unit (OTU) was generated in iTol (Letunic and Bork, 2007).

The relationship of environmental factors and the species composition of community (based on the relative abundance of top 250 OTUs) were analyzed by the constrained linear ordination technique redundancy analysis (RDA) using CANOCO v4.5 (Microcomputer Power, USA) and the BIOENV analysis provided in PRIMER 5 software (Primer-E Ltd, UK). RDA was used to test which of the environmental factors explain the majority of the variation in the bacterial/archaeal community composition (Monte Carlo permutation test, 1000 permutations) and show relationship between major bacterial families and environmental factors. The BIOENV analysis was applied to determine the correlation between the environmental factors and bacterial/archaeal communities with the application of a Spearman's correlation coefficient.