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Community composition and distribution of sulfate- and sulfite-reducing prokaryotes in sediments from the Changjiang estuary and adjacent East China Sea

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ABSTRACT

Sulfate- and sulfite-reducing prokaryotes (SSRP) communities play a vital role in both sulfur and carbon cycles. Community composition and abundance of SSRP were investigated using dissimilatory sulfite reductase β subunit (*dsrB*) gene sequencing in sediments from the Changjiang estuary and its adjacent area in the East China Sea (ECS). Clone libraries were constructed and real-time fluorescence quantitative polymerase chain reaction (qPCR) was applied to understand the community information of SSRP. In addition to sequences affiliated to sulfate-reducing prokaryotes (SRP), those affiliated with sulfite-reducing prokaryotes (SiRP) were also observed. Four phylotypes of SRP in this study showed genetic similarity to Desulfobulbaceae, Syntrophobacteraceae, Desulfobacteraceae and Peptococcaceae, and an unknown group that could not be clearly affiliated with known lineages was found. Salinity, temperature and contents of total organic carbon (TOC) were most closely correlated with the SSRP communities by canonical correspondence analysis (CCA). ^{210}Pb activities demonstrated the sedimentary environment at S33 was more stable than that at S31. Intense resuspension and reconstruction of sediments made the vertical abundance profile of SSRP fluctuate violently. For surface sediments, the *dsrB* gene copy numbers near the Changjiang estuary were higher than those in the mouth of Hangzhou Bay and the mud deposits along the Zhejiang coast, and contents of TOC were positively related to the copy numbers of *dsrB* gene. Our data provided valuable information to achieve a better understanding of the potential role of SSRP in sediments from the Changjiang estuary and adjacent East China Sea.

1. Introduction

Sulfate reduction, the sequential reduction of sulfate to sulfite and then to sulfide, plays a vital role in the global sulfur cycle and organic matter mineralization, and it could even account for up to 50% of the total organic carbon (TOC) degradation in some reduced environments (Wang et al., 2008; Fan et al., 2012). Sulfate reduction is mediated by sulfate-reducing prokaryotes (SRP), a group of microbes with great morphological diversity and variation in nutrition types. In anaerobic environments, such as estuarine sediments, SRP are major contributors to carbon and sulfur cycle (Pérez-Jiménez and Kerkhof, 2005). Using sulfate as a terminal electron acceptor, SRP constitute a paraphyletic group of physiologically diverse anaerobes, which all share the ability to obtain energy from dissimilatory reduction of inorganic sulfate (Joulian et al., 2001; Nakagawa et al., 2004). SRP are important organisms in environments, especially for the degradation of organic matter in coast and also in deeply buried marine sediments in the open ocean (Blazejak and Schippers, 2011).

In recent years, many researchers have investigated the community composition and diversity of SRP in diverse environments, and marine sediment has drawn worldwide attention (Fukuba

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et al., 2003; Gillan et al., 2005; Blazejak and Schippers, 2011). Among these investigations, dissimilatory sulfite reductase gene (*dsr*) has been widely used as a molecular marker. Dissimilatory sulfite reductase (DSR) catalyzes the six electrons reduction of sulfite to form sulfide, the final and essential step in the anaerobic sulfate respiration pathway (Minz et al., 1999; Klein et al., 2001; Neretin et al., 2003). DSRs have an $\alpha_2\beta_2\gamma_2$ or $\alpha_2\beta_2$ structure, possess iron-sulfur clusters and siroheme prosthetic groups (Cottrell and Cary, 1999). The functional marker genes *dsrA* and *dsrB* encode the two major subunits α, β of dissimilatory sulfite reductase (Kjeldsen et al., 2009). *Dsr* genes are highly conserved, which make this enzyme ideal for assessing the community information of SRP in nature (Kondo et al., 2008).

Culture-independent molecular methods provide more convenient and accurate approaches for microbial ecology study (Dang et al., 2010). 16S rRNA gene are often used as the molecular marker, while PCR primers targeting 16S rRNA genes may cross-react with members of other groups, especially when used in environmental samples containing complex microbial gene pools (Rotthauwe et al., 1997). Furthermore, 16S rRNA gene-based monitoring of SRP is particularly difficult because known SRP belong to several diverse and distant lineages and, therefore, a large number of primers or probes are needed to cover entire SRP communities specifically (Lücker et al., 2007). Besides SRP, highly conserved functional gene *dsrAB* was found in sulfite-reducing prokaryotes (SiRP) and sulfide-oxidizing prokaryotes (SOP) (Fan et al., 2012). *DsrAB* gene was mainly possessed by SRP and SiRP, and *dsrAB* phylogeny presents the oxidative-operating sulfite reductases of SOP distantly related to the reductive enzyme types of SRP (Meyer and Kuever, 2007).

Located offshore from the mouth of the Changjiang River, the Changjiang estuary acts as a filter between land and ocean and is the interface of fresh water and saline water (Chen et al., 1999; Feng et al., 2009). As the longest river in China, the Changjiang River contributes significant nutrients into the estuary and sea annually. The high organic substance could promote rapid diagenetic processes in the water–sediment interface, affecting the biogeochemical cycling of carbon, nutrients and many other chemical elements in coastal oceans (Hedges et al., 1999). With an average water depth of 60 m, the biogeochemical processes in the East China Sea (ECS) are greatly affected by inputs from the Changjiang River and several along-shelf and cross-shelf currents (Liu et al., 2003a). Owing to its specific location, the Changjiang estuary and its adjacent areas of the ECS show a high bacterial diversity (Feng et al., 2009). Under anaerobic conditions, SRP play a key role in the degradation of organic substances. However, to our knowledge, only a few studies have examined the diversity and abundance of SRP communities in the Changjiang estuary and its adjacent area in the ECS. For example, Nie et al. (2009) used polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) method to investigate the community composition of SRP in rhizosphere soils in the Changjiang estuary, and Wu et al. (2009) used clone library and DGGE methods to understand the community diversity of SRP in the aquifer underlying the Laogang Landfill along the shore of the ECS.

In previous studies, many researchers used the 1.9-kb *dsrAB* gene fragment to evaluate SRP communities. This study is based on a shorter sequence 350-bp *dsrB* gene to analyze sulfate- and sulfite-reducing prokaryotes (SSRP) communities, which has shown that the general topology of different lengths of *dsrAB* remains consistent (Pérez-Jiménez and Kerkhof, 2005), and *dsrB* gene has been used successfully as a molecular marker to get the community information of SRP (Cottrell and Cary, 1999; Chang et al., 2001; Geets et al., 2006; Kondo et al., 2012). Additionally, being a shorter sequence, *dsrB* gene is suitable for qPCR quantification. Unlike previous studies, this study is the first to discuss both the community composition and abundance of SSRP in sediments from the Changjiang estuary and its adjacent area in the ECS, using *dsrB* gene sequences. The community composition of SSRP was revealed on the basis of clone libraries, and the abundance of SSRP was displayed quantifying the *dsrB* gene copy number with real-time fluorescence quantitative polymerase chain reaction (qPCR). Our primary objectives were (i) to illuminate the community diversity and abundance of SSRP in our studied area, (ii) to investigate potential links between community composition, abundance of SSRP and environmental parameters.

2. Materials and methods

2.1. Sample collection

Samples were collected from the Changjiang estuary and its adjacent area during 29 July–4 August 2011 by the R/V *Run-Jiang* (Fig. 1). Surface sediments were obtained from all sampling stations using the box corer. Gravity cores (15 cm internal diameter) obtained from station S31 and station S33 were sliced at 1-cm intervals for the first 20 cm and at 2-cm intervals thereafter. Porewater was extracted with the syringe method (Ankley and Schubauer-Berigan, 1994) before the gravity cores were sliced. After collection, all sedimentary samples were placed into sterile ziplock plastic bags, and immediately frozen at −80 °C for nucleic acid extraction.

2.2. Determination of environmental parameters

At each station, environmental parameters (temperature, salinity and dissolved oxygen concentrations) in the bottom water were recorded with a RBR XR-620 Multi-Channel CTD (Elcee, Malaysia) in situ. Other parameters were measured in the laboratory. A QuAAtro nutrient auto analyzer (Seal Analytical Ltd, UK) was used to measure the concentrations of ammonium, nitrite and nitrate in the bottom water from all sampling stations. The contents

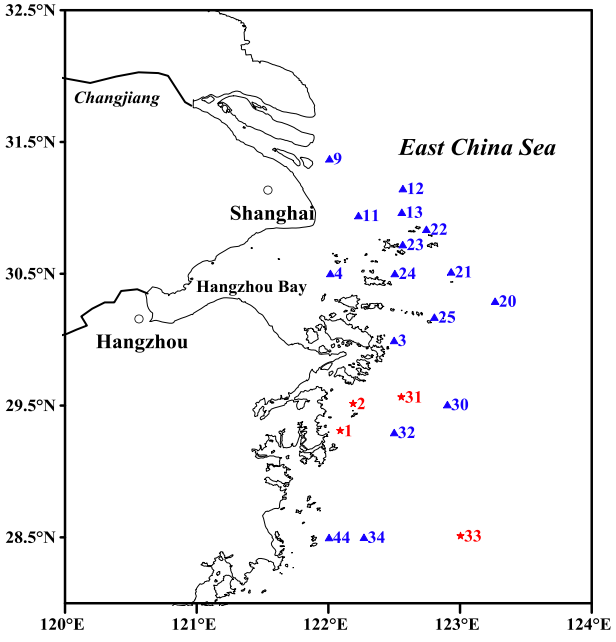

Fig. 1. Sampling stations in the Changjiang estuary and its adjacent area in the ECS. All sampling stations were used for qPCR measurements, and stations marked with ★ were used for establishing clone libraries.