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Microbial diversity in the deep-sea sediments of Iheya North and Iheya Ridge, Okinawa Trough

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ABSTRACT

In this study, we analyzed the bacterial and archaeal diversities of the deep-sea sediments in Iheya North and Iheya Ridge, Okinawa Trough, using the high-throughput sequencing technology of Illumina MiSeq 2500 platform. Four samples (IN1, IN2, IR1 and IR2) were used in this study, of which IN1 and IN2 were located at regions close to and distant, respectively, from the active hydrothermal vents in Iheya North, while IR1 and IR2 were located at regions close to and distant, respectively, from the active hydrothermal vents in Iheya Ridge. The four samples were rich in different metal elements. Sequence analysis based on the V3–V4 regions of 16S rDNA gene obtained 170,363 taxon tags, including 122,920 bacterial tags and 47,433 archaeal tags, which cover 31 phyla, 50 classes, 59 orders, 87 families, and 138 genera. Overall, the microbial communities in all samples were dominated by bacteria, in which *Proteobacteria* was the largest phylum, followed by *Chloroflexi*, *Firmicutes*, *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, and *Nitrospirae*, which together accounted for 64.6% of the total taxon tags. In contrast to the high bacterial diversities, the archaeal diversity was low and dominated by *Thaumarchaeota*, which accounted for 22.9% of the total taxon tags. Comparative analysis showed that (i) IN2 and IR2 exhibited more microbial richness than IN1 and IR1, (ii) IR1 and IR2 exhibited higher microbial diversities than IN1 and IN2, (iii) samples from Iheya Ridge and Iheya North fell into two groups based on principle component analysis. Furthermore, microbes potentially involved in sulfur, nitrogen, and metal metabolism and cycling were detected in all samples. These results provide for the first time a comparative picture of the microbial diversities in the sediments of Iheya North and Iheya Ridge and indicate that geological features and distance from active hydrothermal vents likely play important roles in the shaping of microbial community structure.

1. Introduction

In order to study the diversity of microorganisms associated with the extraordinary environments of deep sea, various culture-dependent and culture-independent methods have been developed, whereby microbial communities in extreme environments, including deep-sea hydrothermal vents, have been discovered (Takai and Nakamura 2011). Compared to culture-dependent techniques, culture-independent methodologies have the advantage of detecting the diversity of the entire microbial system, including that of yet-to-be cultured microorganisms (Campbell et al., 2013; Sylvan et al., 2012a; Takai and Nakamura, 2011; Yanagawa et al., 2013). Culture-independent methods are especially valuable for the study of archaea, of which most lineages derived from deep-sea extreme environments have not yet been cultivated, and little information is available about their physiological and metabolic properties (Takai and Nakamura, 2011).

Okinawa Trough is a back-arc basin located behind the Ryukyu trench and Ryukyu Islands; it contains several active hydrothermal fields, including those in Iheya North and Iheya Ridge. In Iheya North, the hydrothermal field is located in the western peak of the Iheya North Knoll within a restricted area about 250 m in diameter, which was first discovered in 1995 by a deep-sea camera survey (Glasby and Notsu, 2003). Several hydrothermal mounds with vents and diffusing flows have been recognized, including North Big Chimney (NBC), Central Big Chimney (CBC), and Event 18 (E18) (Glasby and Notsu, 2003). The vent fluids in Iheya North are high in alkalinity and abundant in carbon dioxide, hydrogen sulfide, and methane (Ishibashi et al., 1995). The temperature and flow rates of the vent fluid are highest within NBC and generally

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decrease as the distance from NBC increases (Kawagucci et al., 2011). The Iheya Ridge hydrothermal field, which is about 25 km north of the NBC, was first discovered during the three dives of the Shinkai 2000 in September 1988 (Ishibashi et al., 1995). This area is characterized by a thick blanket of muddy sediments consisting of volcanic sands with disseminated minor sulfide (Sakai et al., 1990). In previous studies, bacteria and archaea have been isolated from the hydrothermal fields in Iheya North (Lu et al., 2001; Nagahama et al., 2006; Takai et al., 2002) and Iheya Ridge (Inagaki et al., 2004; Nakagawa et al., 2005b; Takai et al., 2006), and the microbial communities in hydrothermal samples of Iheya North field have also been investigated (Suzuki et al., 2004; Yanagawa et al., 2014); however, very little research on the microbial community in the Iheya Ridge field has been documented (Takami et al., 1999).

The primary goal of this study was to examine the microbial diversities of four sediments located at different regions in Iheya North and Iheya Ridge. For this purpose, the V3–V4 regions of 16S rDNA gene were sequenced via Illumina MiSeq 2500 platform, which is a high-throughput sequencing technology that has recently been frequently used to study microbial diversity in various environments (Moreau et al., 2014; Staley et al., 2013; Sun et al., 2014; Walujkar et al., 2014). However, to our knowledge, we are the first to use this technology for the analysis of biodiversity in Okinawa Trough.

2. Materials and methods

2.1. Sample sites and collection

The samples used in this study were collected at April 16–24, 2014 during the cruise conducted by the scientific research vessel KEXUE in Okinawa Trough. Sediment samples were collected using electro hydraulic grab with underwater television camera (for samples in IR1 and IN1) and box sampler (for samples in IR2 and IN2). The positions of the samples were as follows: IN1 (126°54.32′ E, 27°48.47′ N, ~1190 m), IN2 (126°58.89′ E, 27°48.12′ N, ~1330 m), IR1 (126°58.36′ E, 27°33.07′ N, ~1387 m), and IR2 (126°55.59′ E, 27°34.01′ N, ~1589 m) (Fig. 1). The samples were divided into aliquots and stored at −80 °C immediately. Sediments from 5 to 10 cm under the surface of each sample were used in the study.

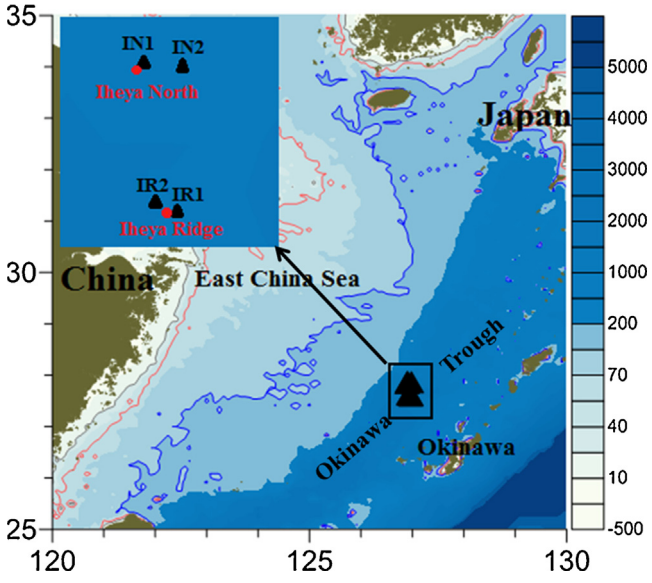

Fig. 1. Location of hydrothermal fields and sampling sites in Okinawa Trough. Map was generated using Surfer 10.0.

2.2. Chemical analysis

Chemical analysis was performed by the Research Center of Analysis and Measurement, Institute of Oceanology, Chinese Academy of Sciences. The samples were evaporated in a drying oven and grounded into powder. Five milliliter of HF, 2 ml HNO_3, and 1 ml $HClO_4$ were added to 0.05 g grounded sample to obtain a total digestion of the particles. An equal volume of HNO_3 was added to the mixture, which was followed by adding ultrapure water to make a total volume of 25 ml. The mixture was then analyzed with Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (PerkinElmer, USA).

2.3. DNA extraction and PCR amplification

Total genomic DNA from the four samples was extracted using FastDNA SPIN Kit (MP Biomedicals, Santa Ana, USA) according to the manufacturer's instructions. DNA concentration was determined using NanoDrop ND-2000 (Thermo Scientific, Wilmington, USA). DNA was diluted to 1 ng/μl in sterile water. The universal primer set 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′) was used for amplification of the V3–V4 regions of 16S rDNA. The PCR reaction was carried out in a 30 μL reaction volume with 15 μL Phusion High-Fidelity PCR Master Mix (New England Biolabs, USA), 0.2 μM forward and reverse primers, and 10 ng template DNA. Thermal cycling consisted of denaturation at 97 °C for 1 min, followed by 30 cycles of 97 °C for 10 s, 50 °C for 30 s, and 72 °C for 60 s, and finally 72 °C for 5 min. The PCR products were analyzed on 2% agarose gel, and the DNA between 380 bp to 440 bp were purified with GeneJET Gel Extraction Kit (Thermo Scientific, Waltham, USA).

2.4. 16S rDNA library preparation and sequencing

Sequencing libraries were labeled with different multiplex indexing barcodes using NEB Next Ultra DNA Library Prep Kit for Illumina (New England Biolabs Inc., USA) following manufacturer's recommendations. The quality of the libraries was assessed with Qubit 2.0 Fluorometer (Thermo Scientific, Waltham, USA) and Agilent Bioanalyzer 2100 system. The libraries were sequenced on an Illumina MiSeq 2500 platform at Novogene (Beijing, China). Complete data with 387 bp, 430 bp, and 404 bp reads had been submitted to the NCBI Short Read Archive database under accession no. SRX878094.

2.5. Quality filtering, operational taxonomic units (OTUs) picking, and annotation

The raw data sequences were assigned to individual samples by their unique barcodes. The 16S rDNA primers and barcodes were then removed to generate pair-end (PE) reads. Raw tags were then generated by merging PE reads with FLASH (Magoc and Salzberg, 2011); the raw tags were then filtered and analyzed using QIIME software package (Quantitative Insights Into Microbial Ecology) (Bokulich et al., 2013). Reads from all samples were quality filtered using an average quality value of 20 (Q20) during demultiplexing; sequences with a mean quality score <20 were excluded from analysis, and chimeras were also excluded. For species analysis, sequences with ≥97% similarity were assigned to the same OTUs using Uparse v7.0.1001 (Edgar, 2013), and similarity hits below 97% were not considered for classification purpose. A representative sequence of each OTU was picked out and the taxonomic information was annotated using RDP classifier (version 2.2) (Wang et al., 2007) and GreenGene database (DeSantis et al., 2006).