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Fungal community analysis in the deep-sea sediments of the Pacific Ocean assessed by comparison of ITS, 18S and 28S ribosomal DNA regions

Wei Xu [a,b,1], Zhu-Hua Luo [a,b,*,1], Shuangshuang Guo [a,b,c], Ka-Lai Pang [d]

[a] Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, 178 Daxue Road, Xiamen 361005, People's Republic of China
[b] Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources, 178 Daxue Road, Xiamen 361005, People's Republic of China
[c] College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, People's Republic of China
[d] Institute of Marine Biology and Center of Excellence for the Oceans, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 20224, Taiwan, ROC

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ABSTRACT

We investigated the diversity of fungal communities in 6 different deep-sea sediment samples of the Pacific Ocean based on three different types of clone libraries, including internal transcribed spacer (ITS), 18S rDNA, and 28S rDNA regions. A total of 1978 clones were generated from 18 environmental clone libraries, resulting in 140 fungal operational taxonomic units (OTUs), including 18 OTUs from ITS, 44 OTUs from 18S rDNA, and 78 OTUs from 28S rDNA gene primer sets. The majority of the recovered sequences belonged to diverse phylotypes of the Ascomycota and Basidiomycota. Additionally, our study revealed a total of 46 novel fungal phylotypes, which showed low similarities (< 97%) with available fungal sequences in the GenBank, including a novel Zygomycete lineage, suggesting possible new fungal taxa occurring in the deep-sea sediments. The results suggested that 28S rDNA is an efficient target gene to describe fungal community in deep-sea environment.

1. Introduction

The deep marine subsurface harbors an immense number of microbes, involved in biogeochemical cycling (Orsi et al., 2013). Besides bacteria and archaea, fungi also have been found to be ubiquitous in the ocean and participate in a range of important ecological roles within this environment, such as decomposers, mutualists, and pathogens (Kohlmeyer and Kohlmeyer, 1979; Hyde, 1989; Hyde et al., 1998). Since the first isolation of deep-sea fungi approximately 50 years ago from the Atlantic Ocean at a depth of 4450 m (Roth et al., 1964), our knowledge of the diversity of culturable fungi in deep-sea environment has been furthered by morphology-based surveys (Burgaud et al., 2009, 2010; Le Calvez et al., 2009; Singh et al., 2010, 2012a).

With the development of culture-independent molecular techniques, especially based on the polymerase chain reaction (PCR) and its derived techniques, the presence of fungi has been directly reported from the deep-sea environmental DNA samples. Earlier studies revealed the diversity of deep-sea fungi from various extreme habitats by universal PCR primers for all eukaryotic organisms, and so our knowledge on the diversity of fungi in the deep-sea environments was fragmentary (Lopez-Garcia et al., 2001, 2003, 2007; Edgcomb et al., 2002; Takishita et al., 2007). Until recently, various fungal-specific PCR primers, mainly targeting the 18S and internal transcribed spacers (ITS) rDNA gene regions, have been used to report fungal communities in these environments (Bass et al., 2007; Lai et al., 2007; Le Calvez et al., 2009; Nagano et al., 2010; Nagahama et al., 2011; Singh et al., 2011, 2012a, 2012b; Xu et al., 2014). Nagahama et al. (2011) used two PCR primer pairs to amplify small subunit (SSU) rDNA from deep-sea methane cold-seep sediments at Sagami-Bay (depth at 850–1200 m), identifying a total of 35 phylotypes, 12 of which were early diverging fungi. Singh et al. (2011) investigated the diversity of fungal communities in three different deep-sea sediment samples at a depth of 5000 m; a total of 39 fungal operational taxonomic units (OTUs), with 32 distinct fungal phylotypes, were recovered using three primer pairs (two fungal-specific ITS primer sets and one 18S rDNA primer pair).

Our previous study analyzed the fungal diversity in deep-sea sediments using culture-independent approach by targeting ITS

* Corresponding author at: Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, 178 Daxue Road, Xiamen 361005, People's Republic of China.
E-mail addresses: luozhuhua@hotmail.com, luozhuhua@tio.org.cn (Z.-H. Luo).
[1] These authors contributed equally to this work.

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regions of rRNA genes from five of six locations in this study (Xu et al., 2014). A total of 48 fungal operational taxonomic units (OTUs), with 27 distinct fungal phylotypes, were recovered using six primer pairs (one fungal-specific primer pair and five taxon phylum-specific primer sets). Although the use of fungal-specific primers revealed the presence of diverse fungal phylotypes, including some unknown species, considering that the specific primers designed for amplification of fungal 18S rDNA or ITS regions may be biased towards certain fungal taxonomic groups (Singh et al., 2012b), the diversity of fungal communities in these areas still need to be further studied. Numerous studies suggested that multiple-primer approach would have great potential for obtaining new information on the diversity and ecological role of fungi in the deep sea sediments (Singh et al., 2011, 2012a, 2012b). It is also known that some of the primer pairs designed for amplification of fungal sequences may co-amplify non-fungal templates from environmental samples, leading to inaccurate estimation of fungal diversity (Borneman and Hartin, 2000). As a favored phylogenetic marker among many mycologists, the fungal 28S rDNA gene had not been widely applied to survey fungal diversity (Schoch et al., 2012). This gene was used successfully to detect fungal diversity in environmental samples from various habitats (Rosendahl and Stukenbrock, 2004; Pivato et al., 2007; Porter et al., 2008; Cho et al., 2009), except from deep-sea environment.

In the present study, the diversity of fungi in six deep-sea sediment samples of the Pacific Ocean, with water depths ranging from 5017 to 7068 m, were investigated using three fungal-specific primer sets, i.e. ITS1-5.8S-ITS2, 18S, and 28S rDNA genes. Combined with our previous results of these areas (Xu et al., 2014), we hope that the use of multiple primer sets will generate a more accurate assessment of the fungal diversity in the deep-sea sediments of the Pacific Ocean.

2. Materials and methods

2.1. Sampling and DNA extraction

The deep-sea sediment samples were collected from six locations (water depths ranging from 5017 to 7068 m) in the Pacific Ocean during the cruise of DY115-23 of R/V 'Hai-Yang-Liu-Hao' from June to October 2011, including two samples from the deepest ocean depth, the Mariana Trench. Sampling sites were grouped into three different areas based on their geographic locations: Northwest Pacific Ocean (sites CQ and CW), Central Pacific Ocean (sites W and WS), and Mariana Trench area (sites JK and JL) (Fig. 1). All sediment samples were immediately kept at −20 °C after being collected until used for DNA extraction. Sediment temperature was recorded using a portable electronic thermometer at each site immediately after sediment samples were retrieved on board. The pH and salinity were determined using a FE 20 pH meter (Mettler Toledo, China) and an S-10 hand-held salinity refractometer meter (Atago, Japan), respectively. Details of the collected samples are summarized in Table 1 and Fig. 1.

Genomic DNA was extracted from 0.5 g of sediment sample (wet weight) using a FastDNA® Spin Kit for Soil (MP Biomedicals, USA) according to the manufacturer's protocol. Extractions were performed in triplicate for each sample and DNA extracts were pooled and kept at −20 °C until used. The extracted genomic DNA was quantified by using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA) according to the manufacturer's protocol.

2.2. PCR amplification and clone library construction

PCR amplification was carried out by three primer sets ITS1/4 (ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4: 5′-CGTT`ACTR-

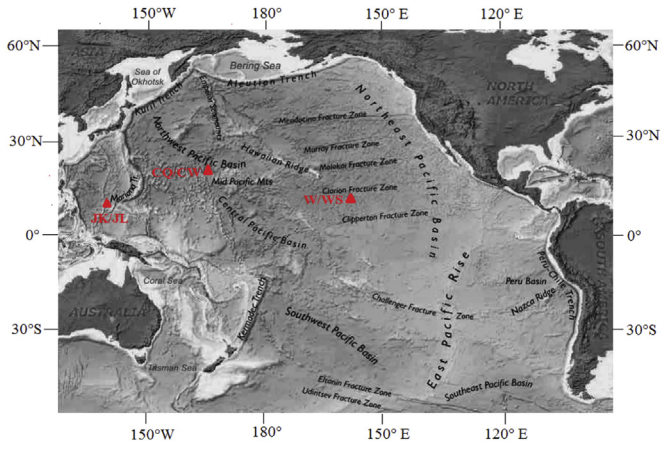

Fig. 1. Map showing the sampling sites in this study.

Table 1
Characteristics of the sediment samples used in this study.

Sample name	Location	Sampling area	Salinity (%)	Depth (m)	pH	*T* (°C)
CQ	171°20′E/ 19°12′N	Northwest Pacific Ocean	3.4	5017	7.36	2–4
CW	170°40E/ 19°50′N	Northwest Pacific Ocean	3.4	5215	7.40	2–4
JK	141°58′E/ 11°00′N	Mariana Trench area	3.4	6986	7.47	2–4
JL	142°23′E/ 11°03′N	Mariana Trench area	3.4	7068	7.50	2–4
W	154°25′W/ 10°00′N	Central Pacific Ocean	3.4	5145	7.48	2–4
WS	154°00′W/ 10°03′N	Central Pacific Ocean	3.4	5062	7.53	2–4

RGGCAATCCCTGTTG-3′), NS1/2 (NS1: 5′-GTAGTCATATGCTTGTCTC-3′ and NS2: 5′-GGCTGCTGGCACCAGACTTGC-3′) and NL1/4 (NL1: 5′-GCATATCAATAAGCGGAGGAAAAG-3′ and NL4:5′-GGTCCGTGTTTCAAGACGG-3′) for fungal ITS, 18S and 28S rDNA regions, respectively (White et al., 1990; O'Donnell, 1993). PCR reaction was carried out with 5 min at 95 °C, 32 cycles of 94 °C for 40 s, 55 °C (for ITS and 18S rDNA) or 53 °C (for 28S rDNA) for 40 s and 72 °C for 1 min, and a final extension of 7 min at 72 °C. The products were separated by electrophoresis in 1% agarose gels, purified using Universal DNA Purification Kit (TIANGEN, China), and quantified with NanoDrop ND-1000 (Thermo Fisher Scientific, USA). Triplicate PCR reaction products were pooled together to minimize PCR bias. The purified fragments were cloned into PGEM-T easy vectors (Progema, USA) and transformed into *Escherichia coli* DH5α. To identify unique positive clone, the inserted DNA sequence was amplified in reaction mixtures by using the vector primers SP6 and T7. More than 100 clones containing positive insert from each clone library were picked for sequencing on an ABI3100 automated sequencer (Applied Biosystems, CA).

2.3. Community structure analysis

Sequences obtained from ITS, 18S and 28S rDNA primers were analyzed separately. All the sequences were checked with Ribosomal Database Project for the presence of chimeras (Cole et al., 2004). These chimeric sequences were eliminated from subsequent analyses, and then the rest of the sequences were analyzed using the Blastn tool (http://www.ncbi.nlm.nih.gov/BLAST/) to get the closest reference sequences. OTUs are defined at sequence groups that differ by 3% using software Mothur (version 1.29.0, USA) (http://www.mothur.org/wiki/Main_Page) by the