Deep-Sea Research I 116 (2016) 22–32

Contents lists available at ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

Molecular diversity and distribution pattern of ciliates in sediments from deep-sea hydrothermal vents in the Okinawa Trough and adjacent sea areas

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ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form
5 July 2016
Accepted 15 July 2016
Available online 16 July 2016

Keywords:
Ciliate diversity
Marine sediments
Hydrothermal vent
Illumina MiSeq platform
Biogeography
Abundant taxa

ABSTRACT

In comparison with the macrobenthos and prokaryotes, patterns of diversity and distribution of microbial eukaryotes in deep-sea hydrothermal vents are poorly known. The widely used high-throughput sequencing of 18S rDNA has revealed a high diversity of microeukaryotes yielded from both living organisms and buried DNA in marine sediments. More recently, cDNA surveys have been utilized to uncover the diversity of active organisms. However, both methods have never been used to evaluate the diversity of ciliates in hydrothermal vents. By using high-throughput DNA and cDNA sequencing of 18S rDNA, we evaluated the molecular diversity of ciliates, a representative group of microbial eukaryotes, from the sediments of deep-sea hydrothermal vents in the Okinawa Trough and compared it with that of an adjacent deep-sea area about 15 km away and that of an offshore area of the Yellow Sea about 500 km away. The results of DNA sequencing showed that Spirotrichea and Oligohymenophorea were the most diverse and abundant groups in all the three habitats. The proportion of sequences of Oligohymenophorea was the highest in the hydrothermal vents whereas Spirotrichea was the most diverse group at all three habitats. Plagiopyleans were found only in the hydrothermal vents but with low diversity and abundance. By contrast, the cDNA sequencing showed that Plagiopylea was the most diverse and most abundant group in the hydrothermal vents, followed by Spirotrichea in terms of diversity and Oligohymenophorea in terms of relative abundance. A novel group of ciliates, distinctly separate from the 12 known classes, was detected in the hydrothermal vents, indicating undescribed, possibly highly divergent ciliates may inhabit this environment. Statistical analyses showed that: (i) the three habitats differed significantly from one another in terms of diversity of both the rare and the total ciliate taxa, and; (ii) the adjacent deep sea was more similar to the offshore area than to the hydrothermal vents. In terms of the diversity of abundant taxa, however, there was no significant difference between the hydrothermal vents and the adjacent deep sea, both of which differed significantly from the offshore area. As abundant ciliate taxa can be found in several sampling sites, they are likely adapted to large environmental variations, while rare taxa are found in specific habitat and thus are potentially more sensitive to varying environmental conditions.

1. Introduction

Deep-sea hydrothermal vents are characterized by their extreme environmental conditions. Taking the hydrothermal vents in the Okinawa Trough as an example, the measured temperature in the vents ranged from 100 °C to 350 °C, with high pressure over 10 MPa, and high hydrogen sulfide ranging from 1.3 to 13.7 mM/kg (Glasby and Notsu, 2003). Hydrothermal vents are inhabited by a specific macrobenthic community that differs significantly from that of adjacent sea areas (Tyler and Young, 2003; Levin et al., 2009). Previous studies also indicate that deep-sea hydrothermal vents encompass very specific and diverse habitats for prokaryotic microorganisms (Kato et al., 2009; Kim and Hammerstrom, 2012). A recent study by Anderson et al. (2015) indicates that abundant and rare bacterial lineages are both geographically restricted in deep-sea hydrothermal vents, whereas archaeal lineages are comparatively different. By contrast, the abundant archaeal lineages are cosmopolitan and similar to those in adjacent sea

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http://dx.doi.org/10.1016/j.dsr.2016.07.007
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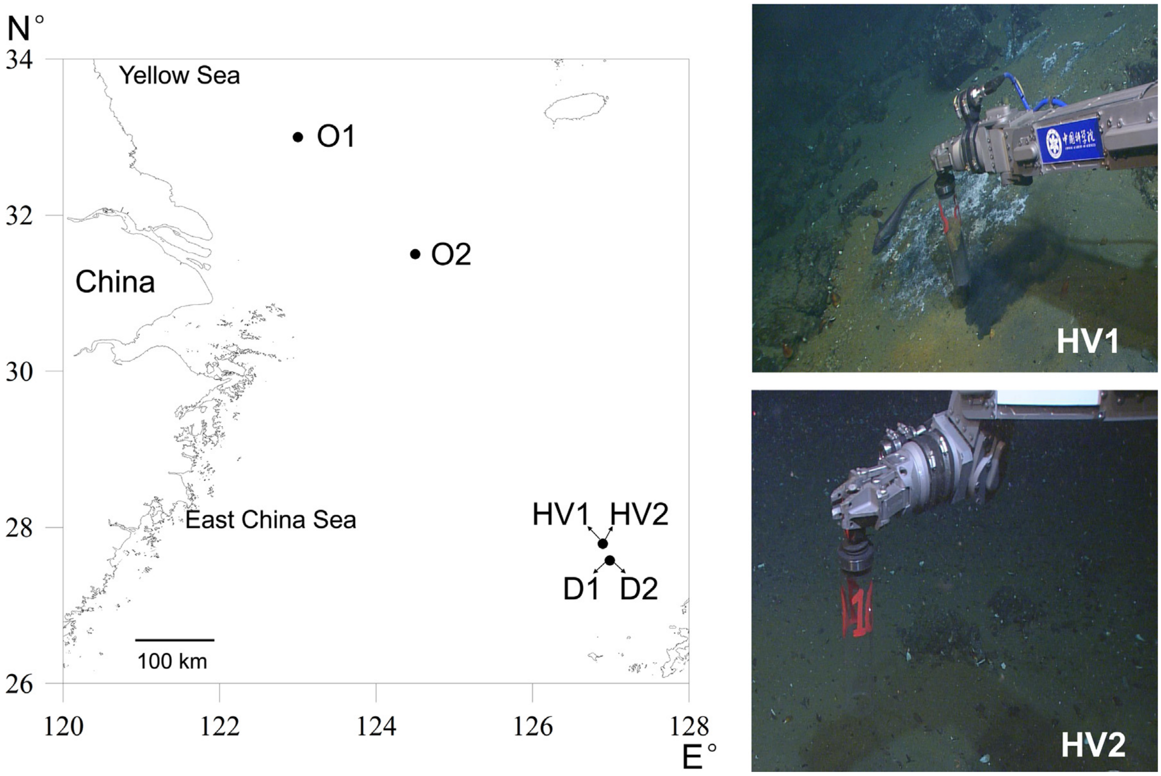

Fig. 1. Location of the six sites and the images of the hydrothermal vent sites HV1 and HV2.

areas, whereas the rare lineages are geographically restricted (Anderson et al., 2015). In comparison with the macrobenthos and prokaryotes, microbial eukaryotes in deep-sea hydrothermal vents have received little attention. Whether microbial eukaryotes have similar patterns of diversity and distribution as bacteria or archaea in hydrothermal vents is still not known.

Among the microbial eukaryotes, ciliates are thought to be a predominant group in the hydrothermal vents of the East Pacific Rise, where at least 20 ciliate species have been recorded following observations by microscopy (Small and Gross, 1985). Subsequent studies based on cultivation led to the descriptions of a limited number of ciliates from hydrothermal vents as well as reports on their physiological characterization (Baumgartner et al., 2002). Due to the extreme environmental conditions in which they live, most ciliates in deep-sea hydrothermal vents are difficult to cultivate resulting in underestimation of their diversity using classical methods.

In recent years, molecular techniques based on the 18S rDNA have revealed a high diversity of microbial eukaryotes in deep-sea hydrothermal vent sediments (Edgcomb et al., 2002; Coyne et al., 2013). Ciliates have often been reported as the most diverse group of microbial eukaryotes in different hydrothermal vents (López-García et al., 2003, 2007; Sauvadet et al., 2010). Seven classes of ciliates, as well as a novel deep-sea group, were detected in the Guaymas Basin hydrothermal vent sediments by 18S rDNA clone library (Coyne et al., 2013).

So far, all studies on the molecular diversity of ciliates and other microbial eukaryotes in deep-sea hydrothermal vents have been based on clone library methods. The clone library approach, however, has inherent methodological limitations, including biases in the plasmid ligation step, the relatively high expense and labour-intensity of sequencing sufficient numbers of clones using the Sanger method (Stoeck et al., 2010). The high-throughput sequencing techniques overcome the limitations caused by the ligation steps of the clone library method enabling hundreds of thousands of sequences to be processed simultaneously. It has been shown that DNA can be preserved in marine sediments over time (Coolen et al., 2009). Thus, the microeukaryotic diversity detected by DNA sequencing likely include not only active organisms, but also extracellular DNA, dead cells and resting stages of organisms. By contrast, cDNA surveys have been used to identify the active protist communities and may provide a different picture of biodiversity from that of DNA surveys (Massana et al., 2015). Therefore, diversity estimation based on a combination of DNA and cDNA high-throughput sequencing will help to better understand the diversity of microbial eukaryotes.

In this paper, we combined the high-throughput DNA and cDNA sequencing of 18S rDNA for the first time to reveal the diversity and group composition of ciliates in sediments from hydrothermal vents. Meanwhile, the ciliate diversity in the hydrothermal vents was compared with those from adjacent deep-sea and offshore sea areas, based on the analysis of high-throughput DNA sequencing data. We aim to (i) evaluate the molecular diversity of ciliates in sediments from deep-sea hydrothermal vents, and (ii) to compare the community composition and distribution of benthic ciliates among the offshore, hydrothermal vent and surrounding deep-sea area.

2. Material and methods

2.1. Study sites and sample collection

The Okinawa Trough is a back-arc basin located behind the Ryukyu trench and Ryukyu Islands, and contains several active hydrothermal vents. Two sites in the immediate vicinity of the hydrothermal vents in the Okinawa Trough were chosen for sample collection. Site HV1 (27°47.4487′N, 126°53.8253′E) was about 5 m away from the nearest hydrothermal vent, and site HV2 (27°47.4404′N, 126°53.8223′E) was about 20 m away (Fig. 1). The water depth in this area was 1008 m. Sediment samples of HV1