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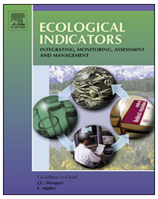

Original Articles

Polyphasic approach revealed complex bacterial community structure and function in deep sea sediment of ultra-slow spreading Southwest Indian Ridge

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ABSTRACT

The Southwest Indian Ridge (SWIR) is one of the least explored mid-ocean ridges and contains a high frequency of hydrothermal vents. We examined the bacterial community structure and metabolic potential of surface sediments collected from a sea mount at 2681 m depth and a ridge-flank at 3776 m in SWIR. High throughput 16S rRNA amplicon sequencing combined with Community Level Physiological Profiling (CLPP) revealed that hydrothermal sediments hosted a diverse microbial community with significant difference in structure and function between seamount and ridge-flank. The ridge-flank community was primarily comprised of facultative chemoautotrophic members of *Burkholderiales* (43.0%) while copiotrophic members of *Alteromonadales* (56.9%) and *Oceanospirillales* (14.6%) were dominant in seamount sample. These results coincided with CLPP of microbial community with higher utilization of carbohydrates such as D-cellobiose, β-methyl-D-glucoside, glucose 1-phosophate and pyruvic acid methyl ester observed in seamount. Culture-dependent approach using array of nutrient media enabled the isolation of heterotrophic genera that exhibited ability to degrade refractory organic matter such as phenanthrene, anthracene, pyrene, fluorene and fluoranthene. These results indicate that bacterial community present in the deep-sea hydrothermal sediment can act as bioreactor for mineralization of refractory organic matter. Thus our study emphasize on the significance of polyphasic approach in comprehensive and rapid assessment of physiological and taxonomic characteristics of the deep seabed bacterial community and further suggested that the bacterial community can act as ecological indicators of the metabolic sources prevalent in the ridge environment.

1. Introduction

At mid-oceanic ridges, chemical and isotopic exchange reactions between the heated seawater and oceanic basement have a profound influence on seawater chemistry (Kastner, 1999; Edmond et al., 1979) and thus effect the mineralogy, geochemistry and physical properties of the oceanic basement. Precipitation of minerals occurs when the hot acid, sulfide and metal-rich fluids mix with cold oxidizing alkaline seawater. The entire ocean volume circulates through oceanic basement at spreading centers in 5–7 million years (Kastner, 1999). Thus, hydrothermal vents play important role in fertilization of oceans with biologically significant minerals such as iron and manganese. Microbial diversity of marine sediments has been studied extensively in the past decades and available knowledge provides a coherent framework for understanding the composition of microbial communities and their *in situ* function. However, our understanding of benthic microbes is strongly skewed towards studies from shallow depths and continental margins. Data sets from open ocean sites and deep sea sediments are scarce, particularly from the mid oceanic ridge systems (Walter et al., 2018; Gold, 1992). Seabed of mid oceanic ridges are characterized with a variety of chemical substances such as manganese, iron, sulphur compounds, methane, hydrogen gas and polycyclic aromatic hydrocarbons (PAH) which form dynamic habitats with distinct chemical gradients that influence the microbial structure (Jannasch and Mottl, 1985; Baker et al., 2013; Yuan et al., 2015). Composition of benthic microbial community in mid oceanic ridges varies with carbon and oxygen content, hydrothermal influence and sediment depth. Oxic surficial sediments harbor relatively diverse bacterial communities with

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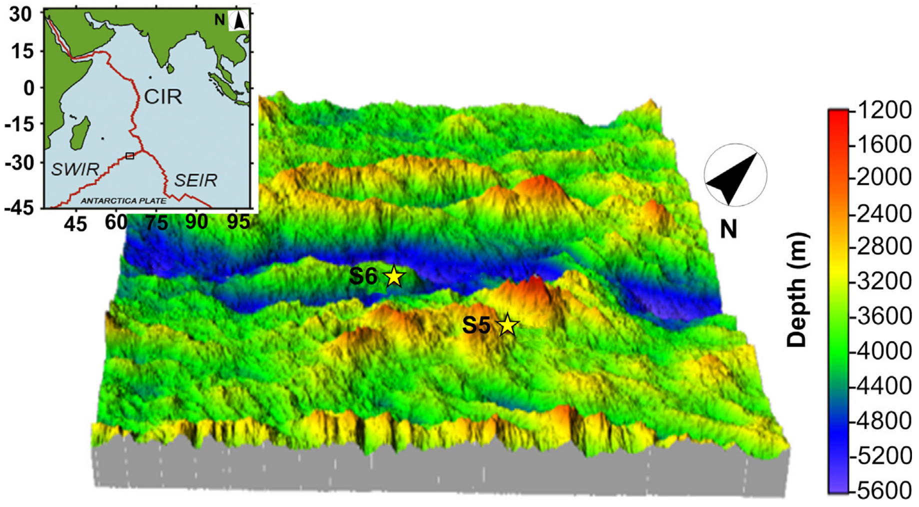

Fig. 1. Geological location of the sampling sites in the Southwest Indian Ridge (SWIR). Also seen are the Southeast Indian Ridge (SEIR) and the Central Indian Ridge (CIR). The black rectangular area on SWIR is enlarged to illustrate the bathymetry of sampling sites at sea mount (S5) and ridge-flank (S6) (☆).

notable representation from the *Alphaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Acidobacteria*, *Actinobacteria* and *Planctomycetes* (Polymenakou et al., 2005; Li et al., 2014; Zhang et al., 2016). In contrast, archaeal diversity in oxic surficial sediment is relatively low with dominant representation from the marine group I *Crenarchaeota.* Deep sea sediments rich in organic matter support bacterial communities dominated by the phylum Candidatus *Atribacteria*, whereas sediments with low levels of organic carbon harbour bacteria related to *Chloroflexi* and *Proteobacteria* (Orcutt et al., 2011). Thermally influenced surficial and deep sea sediments exhibit similar characteristics with increased representation from groups more typically found in hydrothermal environments, such as the *Epsilonproteobacteria* and the *Archaeoglobus/Thermococcus/Methanococcus* groups (Orcutt et al., 2011).

The Southwest Indian Ridge (SWIR) is a boundary between the Antarctic and African plates and extends from the Bouvet triple junction in the Atlantic Ocean to the Rodriguez triple junction in the Indian Ocean. It is one of the least explored, slowest spreading ocean ridges and has diverse geographic environments with an unexpected high frequency of hydrothermal vents (Tao et al., 2012; Tao et al., 2014). Earlier to this discovery, only the Gakkel Ridge was discovered on global ultra-slow spreading ridges (Edmonds et al., 2003). The Gakkel Ridge ranged from 7°W to 86°E (6–11 mm year^{-1}) and has numerous anomalies of the hydrothermal activity like SWIR (Edmonds et al., 2003). In earlier studies at SWIR, arsenite-resistant bacteria belonging to *Proteobacteria* and *Actinobacteria* were isolated and studied from deep-sea sediments (Chen and Shao, 2009). Subsequent culture-independent study using clone library and pyrosequencing revealed that *Thaumarchaeota*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* dominated the archaeal and bacterial communities in SWIR (Li et al., 2014; Cao et al., 2014). Recent high throughput sequencing studies of microbial communities in active and inactive hydrothermal vent location of SWIR has shown that *Epsilon-, Gamma-, Alpha-*, and *Delta-proteobacteria* and members of the phylum *Bacteroidetes* and *Planctomycetes*, as well as *Thaumarchaeota, Woesearchaeota* and *Euryarchaeota* were dominant (Zhang et al., 2016; Ding et al., 2017). Chen et al. (2016) studied the actinobacterial diversity in the deep sea sediments along the SWIR using both 16S rRNA gene pyrosequencing and culture-based methods and demonstrated its diverse potential for degrading recalcitrant organic matter.

Microbial diversity and processes in deep sea sediments are limited by the nature of substrates that are either deposited along with the sediment or that diffuse into it from the overlying seawater or underlying crust (Orcutt et al., 2013) and it varies in its nature and magnitude in contrasting habitats like ridge axis and off-axis sea mounts. Understanding microbial diversity and its metabolic capabilities in oceanic sediments is an important aspect of deep sea research since these microbes shows extraordinary capacity to degrade recalcitrant organic carbon that then circulates back into the ocean and contribute in removal of reduced carbon (Walter et al., 2018). Furthermore, understanding the principles affecting microbial life in environments where chemosynthetic pathways primarily fuel the trophic structure is extremely critical in our understanding of the global oceanic food web structure. To delineate the microbial ecology of distinct sedimentary habitats in the mid-oceanic ridge it is important to consider how microorganisms utilize substrates and gain energy in these environments. Due to its remote location the SWIR is not readily accessible to investigate and very few studies have been conducted to understand these aspects. Therefore, the knowledge on structure and function of microbial community at the SWIR is unclear as there are no studies which involve array of culture-dependent and culture –independent techniques to address these questions.

The aim of this study was therefore to investigate the taxonomic diversity of bacterial community and their organic carbon degradation potential in deep-sea sediments from two different geochemical regimes (ridge flank and off-axis seamount) in the SWIR. For this purpose, community level physiological profiling (CLPP) and bacterial diversity analysis using culture-dependent (using an array of culture media) and culture-independent (using amplicon sequencing of V3 region of 6S rRNA gene) techniques were undertaken. Our study provides a comprehensive understanding of the taxonomic diversity of deep sea-sediment bacterial communities of the ultraslow spreading SWIR, along with the metabolic capabilities of bacterial isolates retrieved from these samples.

2. Materials and methods

2.1. Site description and sample collection

Sediment samples were collected from the two locations on the SWIR using a Van veen's grab on-board *ORV Sagar Kanya* during October 2015 (Fig. 1). Site S5 (26°56′49″S 67°19′47″E) is located at a depth of 2681 m on the slope of a sea mount and site S6 (26°46′08″S 67°43′08″E) is a located on the flank of the ridge axis at a depth of 3776 m. Sediment samples were collected in wide-mouth sterilized polypropylene bottles and stored at 4–5 °C until analysis. For metagenomic analysis, the sediment samples were frozen at −80 °C. Further analysis was performed after bringing the sample to home laboratory at ESSO-NCAOR, Goa, India. Geochemical characteristics of the two samples were determined using inductively coupled plasma mass spectrometry (ICP-MS) as described by Li et al. (2016).