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Microbial diversity in deep-sea sediments from the Menez Gwen hydrothermal vent system of the Mid-Atlantic Ridge

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A B S T R A C T

Deep-sea hydrothermal sediments are known to support remarkably diverse microbial consortia. Culture-independent sequence-based technologies have extensively been used to disclose the associated microbial diversity as most of the microorganisms inhabiting these ecosystems remain uncultured. Here we provide the first description of the microbial community diversity found on sediments from Menez Gwen vent system. We compared hydrothermally influenced sediments, retrieved from an active vent chimney at 812 m depth, with non-hydrothermally influenced sediments, from a 1400 m depth bathyal plain. Considering the enriched methane and sulfur composition of Menez Gwen vent fluids, and the sediment physicochemical properties in each sampled area, we hypothesized that the site-associated microbes would be different. To address this question, taxonomic profiles of bacterial, archaeal and micro-eukaryotic representatives were studied by rRNA gene tag pyrosequencing. Communities were shown to be significantly different and segregated by sediment geographical area. Specific mesophilic, thermophilic and hyperthermophilic archaeal (e.g., *Archaeoglobus*, *ANME-1*) and bacterial (e.g., *Caldithrix*, *Thermodesulfobacteria*) taxa were highly abundant near the vent chimney. In contrast, bathyal-associated members affiliated to more ubiquitous phylogroups from deep-ocean sediments (e.g., *Thaumarchaeota MGI*, *Gamma-* and *Alphaproteobacteria*).

This study provides a broader picture of the biological diversity and microbial biogeography, and represents a preliminary approach to the microbial ecology associated with the deep-sea sediments from the Menez Gwen hydrothermal vent field.

1. Introduction

Deep-sea hydrothermal vents typically occur along back-arc basins and mid-ocean ridges, such as the East Pacific Rise (EPR) or the Mid-Atlantic Ridge (MAR). These ecosystems are supported by chemosynthetic processes, where chemoautotrophic microorganisms are primary producers and organic matter is generated from the oxidation of reduced compounds such as sulfide, hydrogen and methane (Jannasch and Mottl, 1985; Reysenbach and Shock, 2002).

Around the active vents, emanations of thermally charged hydrothermal fluids (a blend of dissolved gases – H_2, CH_4, H_2S, CO_2 – and minerals – mainly composed of silicon, iron, magnesium, zinc) mix with cold oxidized seawater, building up chimney-like mineral deposits (Haymon, 1983). Specialized micro- and macrofauna thrive in these microhabitats. The chemoautotrophic microbial communities are seen as crucial players in the biogeochemical cycling of carbon, nitrogen and sulfur, and are known to comprise both free-living organisms and macrofauna-associated symbionts (Miroshnichenko, 2004; Nakagawa et al., 2005; Yamamoto and Takai, 2011). The deep-sea hydrothermal vents constitute thus important sources of chemical energy, responsible for maintaining chemoautotrophy widespread along the deep-sea floor and for fuelling the oceanic primary and secondary production (Reysenbach and Shock, 2002; Miroshnichenko, 2004; Dick et al., 2013). The energy and carbon sources available for autotrophy are known to depend on the rock source hosting the hydrothermal systems and the chemical composition of its vent fluids (Takai et al., 2006; Amend et al., 2011).

Neighbouring both the Lucky Strike and Rainbow vent fields, the Menez Gwen vent field (37° 51′ N, 31° 31′ W) is a ridge centred, basalt-hosted hydrothermal complex, at 850 m depth. Discovered in 1994 (Fouquet et al., 1994) southwest of the Azores Triple Junction (ATJ), the Menez Gwen remains one of the least investigated vent fields on the northern MAR. Almost all studies to date focused on macrofauna

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(Desbruyères et al., 2001; Martins et al., 2011; Marcon et al., 2013), geochemical (Charlou et al., 2000) and geological (Gale et al., 2011) composition of the vent field. Despite the lack of knowledge regarding its sediments' associated microbial ecology, the Menez Gwen vent field is foreseen as a promising hotspot for microbial diversity studies. A recent study conducted on Menez Gwen diffuse hydrothermal fluids (Winkel et al., 2014) pointed to a prevalence of lithoautotrophic sulfur-oxidizers in these fluids and thus a largely autotrophy-based microbial community.

Most of the microbial surveys conducted in other MAR hydrothermal habitats (Lopez-Garcia et al., 2003; Nercessian et al., 2005; Perner et al., 2007; Voordeckers et al., 2008; Reed et al., 2009; Flores et al., 2011; Roussel et al., 2011), revealed a wider microbial diversity than previously recognized. The chemoautotrophic *Epsilon-* and *Gammaproteobacteria* were identified as being the predominant bacterial colonizers, while the archaeal representatives presumed to account for 33–50% of the total microbial community in these environments (Nercessian et al., 2003). Most archaeal members were described as anaerobic thermophiles and hyperthermophiles, found in the hottest part of the vents, of which some are believed to be transported upwards with hydrothermal fluids from the subseafloor (Deming and Baross, 1993).

The present study aims at investigating the microbial community composition of two sites within the Menez Gwen hydrothermal system. Given the particular geochemical characteristics of sediments in these two different geographic areas, we hypothesized that the microbiota within each sample source would be different. We selected sediment samples from I) a 812 m deep site adjacent to an active vent chimney, under the influence of regular hot hydrothermal fluid emissions, and from II) a 1400 m deep bathyal plain in a volcano slope distant from vent emanations. We assessed the taxonomic profiles of the sediment's microbial communities by means of rRNA gene tag pyrosequencing. Then, we investigated the major microbial biodiversity variations as determined by (i) the use of two different DNA extraction methods and (ii) the geographic location of the two deep-sea sediments. The outcome of this work is the first approach to describe the microbial diversity in marine sediments from two spots within the Menez Gwen hydrothermal vent system. Sampling opportunities from this deep-sea vent field are scarce, limiting thus the information provided from available sediment samples and its natural microbial community structure. Nevertheless, the data here obtained is considered as a preliminary study to further our understanding on the dominant and specialized microbial populations found in the Menez Gwen vent field.

2. Materials and methods

2.1. Study site

The Menez Gwen vent field (37° 51'N, 31° 31′ W) is an offshore hydrothermal complex located in the Mid-Atlantic Ridge, inside the Portuguese EEZ within the Azores Marine Park. Its hydrothermal activity is mainly concentrated over small areas, essentially on a small volcano located at the top central area of the field and whose highest point reaches 800 m below the sea surface (Marcon et al., 2013). The diffuse fluid temperature ranges between 10 and 56 °C while the hottest detected fluid escapes from chimneys at approximately 284 °C. *In situ* pH is acidic (4.2–4.9), and hydrothermal fluids are characterized by strong reduced conditions in relation to seawater, low concentrations of H_2 (<48 μM) and H_2S (<1.8 mM) and high CH_4 concentrations (1.4–2.6 mM) when compared to neighbouring vents (Charlou et al., 2000; Desbruyères et al., 2001; Winkel et al., 2014). Previous surveys of water column samples suggest that methane escaping from the Menez Gwen vents, on the top of the young volcano, is possibly transported 1–2 km north and south by deep tidal currents, following the seafloor morphology and the assumed South–North prevailing direction of currents. Three to four kilometres away from this location, the methane content decreases suggesting that it is diluted in the water column (Borowski et al., 2010).

2.2. Collecting sediment samples

Sediment cores were retrieved from the ocean floor during the DEEPFUN cruise in July 2012, aboard the R/V *Thalassa* using the remotely operated vehicle (ROV) *VICTOR6000* (Ifremer) equipped with a CTD (conductivity–temperature–depth) probe. Two different sites spaced 6.33 km apart were sampled within the Menez Gwen hydrothermal system. One sampling location was chosen in the proximity of an active vent chimney at a depth of 812 m (VC-samples). The other sampling site corresponded to a deeper bathyal plain not affected by vent emanations and at a depth of 1400 m (BP-samples) (Table 1). A blade corer device retrieved the upper 10 cm of the surface sediment, visibly distant from macrofauna or bacterial mats. Due to deep-sea sampling constraints and diving opportunities it was not possible to collect more than one sediment corer from each sampling location. Small-scale heterogeneity was obtained from subsampling the top 5 cm of each sediment corer, which was aseptically divided into 4 subsamples (numbered from 1 to 4). All subsamples were snap-frozen in liquid nitrogen and stored at −80 °C until further DNA extraction.

Table 1
Location and properties of deep-sea sediment samples collected in the Menez Gwen hydrothermal vent field.

	Menez Gwen sediment samples	
	VC	BP
Site location	Vent chimney	Bathyal plain
Sampling date	24-07-2012	25-07-2012
Sampling hour	16:29:10	14:30:28
Latitude	N 37° 50.6696′	N 37° 48.8643′
Longitude	W 031° 31.1584′	W 031° 34.8155′
Depth [m]	812.4	1400.4
pH (strips dipped into the sediment)	4 [4.2–4.9]*	8
In situ temperature [°C]	9.1 [8–284][a]	5.6
Nitrogen saturation [ml/l]	11.6	12.5
Oxygen saturation, Weiss [ml/l]	6.4	7.0
Density [density, kg/m^3]	1031	1034
Conductivity [S/m]	3.8	3.5
Near macrofauna	No	No
Sediment texture	Ash grey fine sediments	Light brown muddy sediments
Sampling	Near an active vent chimney, hydrothermally influenced	Bathyal plain, non-hydrothermally influenced

"VC" and "BP" sites are located 6.33 km away from each other. CTD data, density, nitrogen and oxygen saturation were obtained during sampling from the *in situ* sea water. Post-sampling pH measurement of sediments was performed with test strips.

[a] Values of *in situ* temperature and pH [in brackets] were previously reported (Charlou et al., 2000; Winkel et al., 2014).

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