Deep-Sea Research II 108 (2014) 17–26

Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

Patchiness of deep-sea benthic Foraminifera across the Southern Ocean: Insights from high-throughput DNA sequencing

Franck Lejzerowicz [a,*], Philippe Esling [a,b], Jan Pawlowski [a]

[a] Department of Genetics and Evolution, University of Geneva, Switzerland
[b] IRCAM, UMR 9912, Université Pierre et Marie Curie, Paris, France

ARTICLE INFO

Available online 7 August 2014

Keywords:
Deep-sea sediment
Benthic patchiness
High-throughput sequencing
Foraminifera
Environmental DNA

ABSTRACT

Spatial patchiness is a natural feature that strongly influences the level of species richness we perceive in surface sediments sampled in the deep-sea. Recent environmental DNA (eDNA) surveys of benthic micro- and meiofauna confirmed this exceptional richness. However, it is unknown to which extent the results of these studies, based usually on few grams of sediment, are affected by spatial patchiness of deep-sea benthos. Here, we analyse the eDNA diversity of Foraminifera in 42 deep-sea sediment samples collected across different scales in the Southern Ocean. At three stations, we deployed at least twice the multicorer and from each multicorer cast, we subsampled 3 sediment replicates per core for 2 cores. Using high-throughput sequencing (HTS), we generated over 2.35 million high-quality sequences that we clustered into 451 operational taxonomic units (OTUs). The majority of OTUs were assigned to the monothalamous (single-chambered) taxa and environmental clades. On average, a one-gram sediment sample captures 57.9% of the overall OTU diversity found in a single core, while three replicates cover at most 61.9% of the diversity found in a station. The OTUs found in all the replicates of each core gather up to 87.9% of the total sequenced reads, but only represent from 12.2% to 30% of the OTUs found in one core. These OTUs represent the most abundant species, among which dominate environmental lineages. The majority of the OTUs are represented by few sequences comprising several well-known deep-sea morphospecies or remaining unassigned. It is crucial to study wider arrays of sample and PCR replicates as well as RNA together with DNA in order to overcome biases stemming from deep-sea patchiness and molecular methods.

1. Introduction

Spatial heterogeneity ('patchiness') is a typical feature of deep-sea benthic communities. It is usually linked to sediment characteristics, biogenic disturbances and structures, and the scattered distribution of food resources. All of these factors tend to create microhabitat variations on scales ranging from centimetres to metres (Jumars, 1976; Grassle and Morse-Porteous, 1987; Grassle and Maciolek, 1992; Rice and Lambshead, 1994). Highly patchy distributions have been documented for many deep-sea organisms, including macrofauna (Hessler and Jumars, 1974; Levin et al., 1986; McClain et al., 2011), metazoan meiofauna (Gambi and Danovaro, 2006; Danovaro et al., 2013), and protists such as foraminifera (Bernstein et al., 1978; Bernstein and Meador, 1979; Griveaud et al., 2010). Important variations in deep-sea benthic communities were observed at different spatial scales, ranging from less than a metre to several kilometres (Gambi and Danovaro, 2006; McClain et al., 2012). The variability at local scales was suggested to be responsible for spectacularly rich deep-sea biodiversity (Grassle, 1989; Snelgrove and Smith, 2002). However, this high local variability in species distributions could also act to limit the detection of globally distributed species. For example, *Epistominella exigua* is a deep-sea species that occur in all oceans but not necessarily in every gram of sediment (Pawlowski et al., 2007; Lecroq et al., 2009).

Environmental DNA (eDNA) surveys focusing on the deep-sea floor offer a new insight into the assessment of the influence of the patchiness phenomenon on the distribution of micro- and meiofaunal species. High-throughput sequencing (HTS) generates millions of sequences allowing much more efficient detection of species in the environment (Hajibabaei et al., 2011; Kelly et al., 2014), including rare DNA template molecules preserved in the sediment for millennium (Lejzerowicz et al., 2013a). However, most of the deep-sea eDNA surveys are based on remote, point samples (Scheckenbach et al., 2010; Pawlowski et al., 2011a; Bik et al., 2012). Therefore, their conclusions regarding the macro and meso-scale distribution of deep-sea species have to be taken with

* Correspondence to: Department of Genetics and Evolution, University of Geneva, Sciences 3, 30, Quai Ernest Ansermet, CH-1211 Geneva 4, Switzerland. Fax: +41 22 379 30 77.
E-mail address: franck.lejzerowicz@unige.ch (F. Lejzerowicz).

http://dx.doi.org/10.1016/j.dsr2.2014.07.018
0967-0645/

caution, as it is impossible to evaluate the representativeness of these samples at such a small scale.

To fill this gap, we have performed an eDNA study at multiple scales. We analysed the diversity of deep-sea Foraminifera based on a taxonomic DNA marker sequenced in 42 sediments using PCR enrichment and the multiplexed Illumina HTS (Lecroq et al., 2011, Lejzerowicz et al., 2013a). We sequenced three 1-g surface sediment replicates per sediment core, subsampled consistently from each of the two cores obtained per multi-corer, deployed two to three times in 3 stations. We rely on an extensive reference database existing in our laboratory and partly included in Foram Barcoding project (Pawlowski and Holzmann, 2014) to assign the environmental sequences to taxa already recognized in previous genetic studies of deep Southern Ocean (Gooday et al., 2004a, 2004b; Brandt et al., 2007; Cedhagen et al., 2009; Pawlowski et al., 2007, 2011b). Some traditional studies based on morphological identification provide us with the comparison of foraminiferal patchiness inferred from eDNA data and microscopic counts (Cornelius and Gooday, 2004).

2. Material and methods

2.1. Sampling sites and experimental planning

We collected deep-sea surface sediment samples and subsamples from the 2 February to the 3 March 2012 on board of FS Polarstern during the ANTXVIII/3 expedition to the Southern Ocean (Table 1). The samples were taken with a multi-corer (MUC) equipped with 12-tubes arranged in 2 pairs of perpendicular axes comporting 2 or 4 tubes. Each tube allows the retrieval of a sediment core of 57 mm in diameter, corresponding to 25.5 cm^2 of surface sediment. We recovered abyssal plain sediment cores from three stations, including two neighbour stations located slightly east of the southern end of the Mid-Atlantic Ridge (PS79/86 and PS79/141) and one remote station in the Georgia Basin (PS79/175). We performed two MUC deployments at both Mid-Atlantic stations, and three deployments at the Georgia Basin. From each MUC deployment, two cores with undisturbed surface sediments were selected. From each selected core, we subsampled three sample replicates from the top centimetres of sediment in three 1.5 ml Eppendorf tubes (Fig. 1). Extreme precautions were undertaken to avoid contaminations by wearing gloves all times and using individually packed, sterile disposable spatulas. The samples were stored on board at −80 °C and shipped frozen to the laboratory.

2.2. DNA extraction and PCR amplification

We gently thawed the deep-sea sediment samples on ice and centrifuged the 1.5 ml tubes at 2500*g* for 5 min in order to pellet the deep-sea sediments of high water content. We then discarded the overlaying liquid and, for DNA extraction we weighted approximately 1 g of pelleted sediments using disposable spatulas. We extracted the total DNA content of each gram of sediment using the PowerSoil™ DNA Isolation Kit (MoBio) according to the manufacturer instructions. We performed all extractions in a PCR-free environment, in three separate sessions involving 15, 15 and 12 samples, respectively. We included blank extractions (i.e. tubes without sediment) at a ratio of 1:3, meaning that we generated one control sample each three actual samples (i.e. each set of core pseudo-replicates) in order to monitor for sporadic contamination events. Then, we PCR amplified the foraminiferal-specific 37f hypervariable region of the 18S ribosomal RNA gene from each metagenomic DNA extract using tagged PCR primers for sample multiplexing. Each tagged PCR primer consists of a unique tag sequence of 8 nucleotide appended to the 5′-end of the common amplification primer sequence. None of the 8-nucleotide sequence position is homologous to its corresponding position in the conserved region of the foraminiferal template sequences (absolute anti-complementarity). The minimum pairwise edit distance among forward primer tags and among reverse primer tags is set to 3 differences. No dinucleotide is allowed in any tag, and the base diversity is maximized in order to be able to select tag primer pair combinations increasing the evenness of the anti-complementary bases at each of the 8 sequenced tag positions. Based on these constraints, we generated a set of 26 tagged primer sequences for the forward s14F1 (5′–AAGGGCACCACAAGAACGC–3′) as well as for the reverse foraminiferal-specific primer s15 (5′–CCACCTATCACAYAATCATG–3′). Including the specific primers and the tags, the amplified fragment is of ca. 180 base pairs on average. All tagged primer sequences and their combinations used for the amplification of 42 samples are provided (Supplementary Tables 1 and 2). We performed each PCR in a total volume of 30 µl, including 1 Unit of AmpliTaq Gold DNA polymerase (Applied Biosystems), 1 × of AmpliTaq Gold buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each primer, and approximately 10 ng of metagenomic DNA extract. The reaction conditions consisted of a pre-denaturation step at 95 °C for 7.5 min in order to activate the AmpliTaq polymerase, followed by 50 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension step at 72 °C for 2 min. One PCR blank control without DNA was included at a ratio of 1:1, i.e. for each sample processed in order to control each tagged primer pair combination. All blank controls remained negative throughout the experiments.

2.3. Library preparation and high-throughput sequencing

For each of the 42 samples, we selected one out of the two positive PCRs obtained for library-preparation and Illumina sequencing. First, we purified a total of 10 PCR products using the High Pure PCR Cleanup Micro Kit (Roche) and we quantified them using the fluorometric quantitation method based on the Qubit® dsDNA HS Assay Kit (Invitrogen). We then run an electrophoresis migration of the 10 absolutely quantified products along

Table 1
Samples coordinates.

Station	MUC cast	Date	Latitude	Longitude	Water depth (m)	Number of cores
PS79/86	86-26	02/01/2012	51°58.87′S	12°3.76′	3966.2	2
	86-29	02/01/2012	51°58.78′S	12°1.95′W	3970.8	2
PS79/141	141-10	02/19/2012	51°15.97′S	12°36.94′W	4087	2
	141-11	02/19/2012	51°16.02′S	12°37.12′W	4113.2	2
PS79/175	175-5	03/04/2012	50°46.69′S	39°25.35′W	4154.2	2
	175-6	03/04/2012	50°46.59′S	39°25.33′W	4155.2	2
	175-8	03/04/2012	50°46.60′S	39°25.39′W	4124	2