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Original article

Pyrosequencing analysis of microbial communities reveals dominant cosmopolitan phylotypes in deep-sea sediments of the eastern Mediterranean Sea

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Abstract

The deep eastern basin of the Mediterranean Sea is considered to be one of the world's most oligotrophic areas in the world. Here we performed pyrosequenicng analysis of bacterial and archaeal communities in oxic nutrient-poor sediments collected from the eastern Mediterranean at 1025–4393 m depth. Microbial communities were surveyed by targeting the hypervariable V5–V6 regions of the 16S ribosomal RNA gene using bar-coded pyrosequencing. With a total of 13,194 operational taxonomic units (OTUs) or phylotypes at 97% sequence similarities, the phylogenetic affiliation of microbes was assigned to 23 bacterial and 2 archaeal known phyla, 23 candidate divisions at the phylum level and distributed into 186 families. It was further revealed that the microbial consortia inhabiting all sampling sites were highly diverse, but dominated by phylotypes closely related to members of the genus *Pseudomonas* and Marine Group I archaea. Such pronounced and widespread enrichment probably manifests the cosmopolitan character of these species and raises questions about their metabolic adaptation to the physical stressors and low nutrient availability of the deep eastern Mediterranean Sea.

Keywords: Pyrosequencing; Microbial diversity; Deep-sea sediments; Cosmopolitan species; *Pseudomonas*; Eastern Mediterranean Sea

1. Introduction

Microorganisms are the most abundant and diverse organisms on Earth. However, most of the microbial world remains to be discovered and relatively little is known about the patterns of microbial distribution within and between the planet's major habitat types [1–3]. This holds particularly true for deep-sea sediments (≥1000 m) despite the fact that they represent one of the planet's largest ecosystems, covering about 95% of the total oceanic bottom and 67% of the Earth's surface (e.g. [4]). Indeed, this type of ecosystem has been poorly investigated thus far, since most biogeographic studies have focused on the photosynthetically productive euphotic zone accounting for no more than 10% of the total ocean volume [3].

This is the case for the Mediterranean Sea, where only a few sparse studies have been carried out thus far in benthic "spot" locations in the Cretan Sea, Ionian Sea, Levantine Sea, southern Cretan margin and the Tyrrhenian Sea (e.g. [5] and references therein) as well as in the Levantine continental margins [6]. By applying conventional capillary methods (i.e. classical Sanger-based sequencing), these studies revealed that Mediterranean oxic sediments are highly diverse and dominated by members of *Proteobacteria*, *Acidobacteria*, *Bacteroidetes* and *Planctomycetes*. However, diversity estimates are expected to be even higher when considering that next

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generation sequencing technologies, such as pyrosequencing, enable determination of the most "rare" taxa and provides large quantities of reliable DNA reads.

Pyrosequencing of environmental DNA allows rapid analysis of microbial communities with a much higher throughput than has previously been possible [7]. The enormous sequence data produced by these methods open up new avenues for ecological research by providing detailed semi-quantitative information on microbial community structure [8]. Indeed, this technology has revealed that bacterial diversity in deep-sea waters is tremendous [9]. Recently, Zinger et al. [3] performed a global survey of microbial diversity in seafloor and seawater ecosystems by synthesizing a total of 9.6 million bacterial V6-rRNA amplicons for 509 samples that span the global ocean's surface to the deep-sea floor. They demonstrated that remote seawater habitats harbor similar communities, whereas those in benthic ecosystems display large-scale beta-diversity patterns (i.e. differentiation among microbial habitats). This was attributed to the limited horizontal physical mixing in sea beds compared to seawater.

To further investigate the variability of microbial communities in deep-sea sediments, we performed a survey of microbial diversity in deep-sea basins of the ultra-oligotrophic eastern Mediterranean Sea. This area is a unique environment considered to be one of the most oligotrophic regions in the world, with an average temperature of deep water masses of at least 13.5 °C ([10] and references therein). Thus, it constitutes a model for a deep, relatively warm and highly oligotrophic bathypelagic habitat [11], where the hypothesis of microbial cosmopolitanism (i.e. global occurrence) versus provincialism (i.e. geographically localized occurrence) will be explored.

Here we used tag pyrosequencing of the V5–V6 hypervariable region of the 16S rRNA gene to assess bacterial and archaeal diversity in 35 oxic, deep-sea sediments collected from 1025–4393 m depth in the eastern Mediterranean Sea during seven oceanographic campaigns (Fig. 1). Given the financial and practical difficulties of exploring the deep ocean, the present data constitute a unique set of information that can enhance our knowledge of microbial communities inhabiting the highly oligotrophic deep sea. Moreover, this study intends to serve as a valuable benchmark for an area that is deemed to receive significant pressure in the near future due to a range of anthropogenic activities (e.g. oil exploitation) [12].

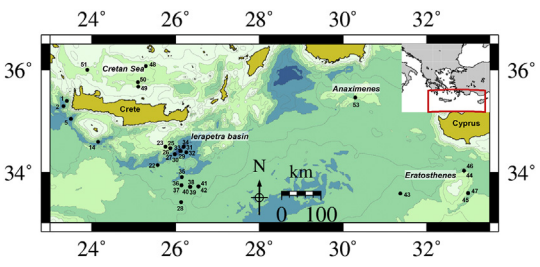

Fig. 1. Map of the eastern Mediterranean Sea showing sampling locations of sediment samples.

2. Materials and methods

2.1. Sampling and chemical analysis

A total of thirty-five ($N = 35$) sediment samples were collected during seven oceanographic surveys that took place between 2006 and 2011 in the eastern basin of the Mediterranean Sea. Samples were collected from the southern Cretan margin (e.g Ierapetra basin; $N = 24$), the Cretan Sea ($N = 4$), the Eratosthenes seamount which is located south of the island of Cyprus ($N = 5$), and the Anaximenes seamount ($N = 2$) (Fig. 1; Supplementary Table S1). A Bowers and Connelly Multiple-Corer (8 corers; i.d.: 9.0 cm) was used to retrieve corers with an undisturbed sediment/water interface. The top 0–1 cm layer of the sediments was scooped into 50 mL falcon tubes (with the help of a sterile spatula over flame) and stored at −20 °C until further processing. The concentration of organic pollutants, total organic carbon (TOC), total chlorophyll-a (Chl-a), phaeopigments (Phaeo), and chloroplastic pigment equivalents (CPEs) have been described in detail by Mandalakis et al. [12]. Here we used the same numbering codes of stations as those presented in the paper of Mandalakis et al. [12], followed by the abbreviated name of sampling locations, i.e. SCM for southern Cretan margin, CrS for Cretan Sea, Erat for Eratosthenes seamount and Anax for Anaximenes seamount stations.

2.2. DNA extraction, 16S rRNA gene library construction and sequencing

Total microbial community DNA was extracted from approximately 1 g of material per station by employing the MoBio UltraClean Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following a slightly modified protocol of the manufacturer, where the bead-beading step was performed in a tissue lyser for at least 30 min (frequency at 30 l/s; TissueLyser II, Qiagen). DNA extracts from replicate samples (2–3 per station) were pooled together and used as templates for library construction. DNA concentrations were quantified using the NanoDrop ND-1000 UV–Vis spectrophotometer (NanoDrop Technologies, USA), and the V5–V6 region of the 16S rRNA gene was amplified by PCR. The PCR reaction mixture (final volume 15 μl) contained 5 μl of 5X KAPA HiFi Fidelity buffer (contains 2.0 mM Mg^{2+} at 1X), 0.75 μl of KAPA dNTP Mix (10 mM each dNTP), ~10 ng of template DNA and 0.50 μl of KAPA HiFi HotStart DNA polymerase (1 U μl^{-1}) (KAPA Biosystems). The V5–V6 region was amplified with universal primers 802f (5′-GATTAGATACCCBNGTA-3′) (reverse complement of primer 802r designed by Claesson et al. [13]) and 1027r (5′-CGACRRCCATGCANCACCT-3′) [13]. Although these primer sets were initially designed to match bacteria, they can also amplify archaeal 16S rRNA genes. The following thermal cycling program was applied: initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 98 °C for 20 s, primer annealing at 55 °C for 15 s and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. Quantification of the PCR