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Archaeal populations in two distinct sedimentary facies of the subsurface of the Dead Sea

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ABSTRACT

Archaeal metabolism was studied in aragonitic and gypsum facies of the Dead Sea subsurface using high-throughput DNA sequencing. We show that the communities are well adapted to the peculiar environment of the Dead Sea subsurface. They harbor the necessary genes to deal with osmotic pressure using high- and low-salt-in strategies, and to cope with unusually high concentrations of heavy metals. Methanogenesis was identified for the first time in the Dead Sea and appears to be an important metabolism in the aragonite sediment. Fermentation of residual organic matter, probably performed by some members of the *Halobacteria* class is common to both types of sediments. The latter group represents more than 95% of the taxonomically identifiable *Archaea* in the metagenome of the gypsum sediment. The potential for sulfur reduction has also been revealed and is associated in the sediment with EPS degradation and Fe–S mineralization as revealed by SEM imaging. Overall, we show that distinct communities of *Archaea* are associated with the two different facies of the Dead Sea, and are adapted to the harsh chemistry of its subsurface, in different ways.

1. Introduction

The Dead Sea as we know it today is a remnant water body of the previous Neogene ingression of the Mediterranean Sea into the Jordan-Arava Rift Valley (Zak, 1967). The so-called Sedom lagoon yielded numerous evaporite deposits in the area and finally disconnected from the sea. Waters originating from this lagoon have evolved within this framework, towards today's well known Mg–Ca–Cl brines of the Dead Sea, due to interactions with the surrounding geology, and through various dissolution and evaporation processes (Zak, 1967; Stein et al., 2000; Katz and Starinsky, 2009). The Dead Sea is now lying 427 m below sea level (2013) and its continuous retreat since 1950 (Katz and Starinsky, 2009) has led to an extreme salinity (TDS) of 348 $g \cdot L^{-1}$ (Oren and Gunde-Cimerman, 2012). In addition, the high concentrations of chlorine and divalent cations in the lake (~6.1 M Cl^-, ~2 M Mg^{2+} and ~0.5 M·Ca^{2+}; Ionescu et al., 2012) make it harsher for microbes to develop (Oren, 2001), approaching MgCl concentrations of 2.3 M which are thought to be the upper limits for life (Hallsworth et al., 2007). While the Dead Sea chemistry is heading towards unfavorable conditions for life (Oren, 2010a), its history is different and life has been observed to thrive when sufficient dilution of the water occurs. One such case is the outflow of submarine springs on the western shore of the lake, where a diverse microbial community has taken advantage of nutrient and salinity gradients to develop (Ionescu et al., 2012; Häusler et al., 2014). In other cases, during events of rainy winters, water dilution in a mixed upper layer has also led to blooms of the alga *Dunaliella* and subsequently of its archaeal degraders of the *Halobacteria* class (Oren and Shilo, 1982; Oren, 1983b, 2010a). During more arid periods, studies have shown that the Dead Sea water column biota consists of halophilic *Archaea* from the *Euryarchaeota* phylum (Oren, 1993; Oren and Ventosa, 1999; Bodaker et al., 2010). Variations in evaporation/precipitation ratio impact the lake physics and chemistry, subsequently influencing the biodiversity of the lake, as well as its geological record, through changes in authigenic precipitation of minerals. These effects are currently investigated in depth within the project of building a water connection between the Red Sea and the Dead Sea (Oren et al., 2004; Bardavid et al., 2007; Abu Qdais, 2008).

In the framework of the paleoenvironmental investigation carried out within the ICDP-sponsored Dead Sea Deep Drilling Project (DSDDP), we were interested in recovering geomicrobiological information on the lake's subsurface, particularly on the potential of microbes to interact with and influence geochemical proxies used for paleoenvironmental reconstructions. Emphasis was put on current subsurface communities, their adaptation to their surrounding and their potential metabolism in such hypersaline conditions. Investigations of *Archaea* communities in the water column and what influences their

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development have been carried out previously (Bodaker et al., 2010; Oren, 1983b). In contrast, there is currently no data on the contribution of these *Archaea* to the subsurface of the Dead Sea. This is of particular interest since in the marine subsurface (Biddle et al., 2006; Lipp et al., 2008) and continental saline (Waldron et al., 2007) sediments, *Archaea* have been identified as major contributors to the biomass. Therefore, using metagenomic methods, we address here the metabolic potential and diversity of *Archaea* in the subsurface of the Dead Sea and their putative influence on two of the main sedimentary facies (Neugebauer et al., in review): aragonite-detritus and gypsum (hereafter AD and GY, respectively). These sedimentary facies are linked to major changes in lake physics and chemistry, triggered by climatic changes or important meteorological events. We thus approach our data with an eye on the mechanisms influencing changes in communities, and their response to the water column influence.

2. Methods

The samples originate from ICDP core 5017-1A of the DSDDP expedition. This core was retrieved at coordinates N 31°30′28.98″, E 35°28′ 15.60″ (middle of the Dead Sea) from a depth of 297 m (one of the deepest point of the lake). Sample GY originates from a core catcher, at depth of 90.64 m below lake floor (Table 1). Sampling was done under sterile conditions using autoclaved pre-cut syringes (see Vuillemin et al. (2010) for more details), in a shore geobiology-specialized lab set up especially for the drilling expedition (December 2010). Sample AD was taken from a core interval (2.74 m) during core opening party in June 2011 at GFZ Potsdam. Cores transported to the ICDP facilities at GFZ Potsdam were sawed into halves. One-half was dedicated to picture taking and non-destructive measurements, while the other was saved for immediate sampling in sterile conditions using similar pre-cut syringes. To prevent any sampling of contaminated or oxidized parts, the syringe minicore was taken from the middle of the liner, and the surface part was removed. DNA was then further extracted using a phenol–chloroform extraction protocol modified from (Ionescu et al., 2009). Cells were extracted from 0.5 g of sediment after multiple cycles of PBS rinsing, 30 s sonication and quick centrifugation. They were then incubated for 20 min at 95 °C in 0.5 mL lysis buffer of 0.1 M Tris, 50 mM EDTA, 100 mM NaCl and 1% SDS (pH 8). 250 μL of phenol:chloroform: isoamyalcohol was added and the samples centrifuged at maximum speed after incubation for 10 min at room temperature. Supernatant was extracted again with the same process and the upper phase collected using Phase Lock Gel TM (PLG) tubes (5 Prime). It was then cleaned twice with 0.5 mL 24:1 (v:v) chloroform: isoamylalcohol and DNA was precipitated overnight at −20 °C in 1 volume of isopropanol and 2% (final volume) of 3 M sodium acetate (pH 5.5). Pellets were washed with 0.5 mL of 70% ice cold ethanol after a 30 min centrifugation at maximum speed, dried and finally dissolved in 10 μL molecular grade water. The QIAEX II Gel Extraction Kit (Qiagen) was used to further remove salt and purify DNA fragments according to the manufacturer's instructions. DNA extracts obtained from samples AD (aragonite alternating with detritus sample) and GY (gypsum sample) were sent to MR DNA™ lab, at Shallowater, Texas for whole genome amplification (through MDA) and metagenomic sequencing. DNA quantification was realized with the Qubit® dsDNA HS Assay Kit (Life Technologies) and amplification performed using the REPLI-g Midi Kit (Qiagen). Enzymatic fragmentation was carried out for 150 ng of each sample using the Ion Xpress Plus gDNA Fragment Library Preparation Kit (Life Technologies). Fragments of 200–300 bp were obtained from the Ion Shear reaction after 8 min in a 37 °C bath. After purification, Ion Xpress Barcode Adapters (Life Technologies) 13 and 14 were ligated to samples AD and GY respectively and underwent nick-repair following the manufacturer's instructions. Size selection at approximately 330 bp was done with the E-gel SizeSelect 2% Agarose Gel (Invitrogen). After new Qubit® determination, fragmented libraries were pooled to an equal DNA amount for each sample to create the final library. The latter was finally diluted to a concentration of approximately 78 pM, bound to Ion Sphere particles using the Ion OneTouch™ 200 Template Kit v2 DL resulting in 16.38% unenriched templated ISPs. After Ion OneTouch ES enrichment, template ISPs were sequenced on an Ion 318 chip using the Ion Torrent PGM (Life Technologies).

Raw sequences were submitted to the MetaGenome Rapid Annotation using Subsystem Technology (MG-RAST) server for annotation and statistical analysis (Meyer et al., 2008). Annotations were made using the M5NR integrative database for proteins and M5RNA for 16S rRNA gene. Classification was made under MG-RAST default settings. Maximum e-value cut-off was 10^{-5}, minimum identity cut-off at 60% and minimum alignment length cut-off of 15. Functions were classified using the Subsystem approach supported by the SEED environment (Overbeek et al., 2005). Statistical values regarding sequence length were obtained from that platform, and by using FastQC (Andrews, n.d.).

The metagenomes are publicly available on MG-RAST under reference IDs 4561562.3 and 4561566.3 for GY and AD respectively.

Diversity indexes were calculated using PAST (Hammer et al., 2001) at the class and genus levels, by excluding unclassified reads within the various phyla. Dominance D is measured as $D = \Sigma((n_i/n)^2)$ where n_i is number of individuals of taxon *i*. Shannon–Weiner Index H is $H = \Sigma((n_i/n)\ln(n_i/n))$ and evenness is taken as Buzas and Gibson's evenness $= e^H/S$, with *S* number of taxa.

Scanning Electron Microscope investigation was done on samples after regular and critical point drying on a Jeol® JSM-7001 FA at the University of Geneva. Energy Dispersive X-ray spectroscopy was carried out on the same device. Samples were mounted on an aluminium stub with double-sided conductive carbon tape. An ultra-thin coating (15 nm) of gold was then deposited on the samples by low vacuum sputter coating.

3. Results

Over the two samples analyzed, 2 147 029 quality-controlled sequences were obtained. Among them, 1 608 197 sequences encoded for 1 008 583 peptides of which to 165 818 at least one annotation was assigned. The sequences had a mean length of 168 ± 63 bp and 171 ± 58 bp (for AD and GY respectively), with modal classes (of over 400 000 sequences for AD and over 900 000 for GY) of 230–239 bp for both samples. Overall, we obtained 128 259 sequences attributed to the domain *Archaea*. The remaining sequences, (bacterial part) will be discussed elsewhere. Sample GY displayed higher number of sequences phylogenetically related to *Archaea* (77 647) but smaller archaeal proportion than AD (20.9% compared with 24.9%, i.e. 50 612 sequences for AD; Table 2; Table S1 in supp. material).

Only few archaeal ribosomal rRNA gene sequences were obtained, therefore, classification using the M5RNA database produced few

Table 1
Sample description and principal geochemical characteristics.
Data for sample AD have been retrieved from Nissenbaum (1975) as expected to be similar to the deep water mass before the 1979 turnover evidenced by the beginning of halite precipitation in the top meters of the core.

Sample	Depth (m)	Lithology	Depositional environment	Salinity (%)	Na^+ (mM)	Ca^{2+} (mM)	Mg^{2+} (mM)	Cl^- (mM)	SO_4^{2-} (mM)
AD	2.74	Alternating aragonite and mud laminae	Startified Holocene Dead Sea	33.21	1726	429	1745	6177	4.2
GY	90.88	Gypsum	End of Pleistocene non stratified Lake Lisan	24.90	1795	74	321	5438	~23