Fluid inclusions from the deep Dead Sea sediment provide new insights on Holocene extreme microbial life

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Abstract

The Dead Sea Deep Drilling Project allowed to retrieve a continuous sedimentary record spanning the two last glacial cycles. This unique archive, in such an extreme environment, has allowed for the development of new proxies and the refinement of already available paleoenvironmental studies. In particular, the interaction of the lake and sediment biosphere with elements and minerals that constitute paleoclimatic proxies has been emphasized. Although life is pushed to its extremes in the Dead Sea environment, several studies have highlighted the impact of microbial activity on this harsh milieu. The paradox is that the identity and means of adaptation of these organisms are largely ignored. We also know relatively little on the way this extreme ecosystem has evolved with time, and how it will react to growing pressure. Constraining this gap should allow to gain precision on the use of paleoenvironmental studies, and also assess the impact of human activity and climate change on a rare ecosystem. In this study, we use halite, the main evaporitic phase during arid periods in the Dead Sea basin and extract ancient DNA from their fluid inclusions, in order to characterize the ancient life of the Holocene Dead Sea. With the aid of an accurately designed protocol, we obtained fossil bacterial and archaeal 16S rRNA gene sequences that illustrate that the main microbial actors of the present Dead Sea have been present in the lake for a relatively long period, emphasizing the stability of this extreme environment. Additionally, we show that current phylotypes of the deep biosphere are present within the obtained fluid inclusions sequences, which would support seeding of the deep biosphere from the water column. Finally, we shed light on putative new actors of the sulfur cycle involving both archaea and bacteria, which could play an unexpected role in the reduction of sulfur species. Together, these data provide new research avenues for both geologists and biologists working in this extreme environment, and help understanding the evolution of the Dead Sea ecosystem with time.

1 Introduction

The goal of every paleo-environmental study is to reconstruct the conditions of life at a given time and in a given space, using a set of direct or indirect indicators, called proxy. One of the targets is often to qualify and quantify the extent of the diversity of a given paleo-ecosystem, and its impact on the environment, in order to analyse its evolution in time, and ideally predicts its future trajectory. Sites of particular interests are those that are today in danger, e.g. in the verge of disappearing, or to be profoundly modified. While these ecosystems become increasingly numerous on our planet, the need for paleo-ecological or paleo-environmental studies, that can be used for comparing with the current states of endangered ecosystems is continuously growing.

Since more than 50 years now, the Dead Sea water budget has been negative, resulting in a massive drop of the shoreline at a rate exceeding one meter per year in the last decade. Halite is almost constantly precipitating and the total dissolved salt concentration has reached the highest level on Earth with up to 348 g. L^{-1} (Oren and Gunde-Cimerman, 2012). The Dead Sea is therefore an extreme ecosystem characterized by a high concentration of divalent cations (~2 M Mg^{2+} and ~0.5 M Ca^{2+}), which makes it a specifically harsh environment for microorganisms that remain the only inhabitants of its water (Oren, 2001). Except for some oases of life located at the outflow of groundwater springs, the number of organism is extremely low in the present Dead Sea water (<10^4; Ionescu et al., 2012). Given the extreme (osmotic) pressure put on organisms, the Dead Sea flora is composed today uniquely of halophilic prokaryotes. The only eukaryote and primary producer in the Dead Sea water is the alga *Dunaliella*, which blooms periodically when the surface layer has been diluted enough by heavy rainfalls over the Jordan catchment area and the surroundings of the Dead Sea (Oren et al., 1995). These events have not occurred since 1992 and the Dead Sea ecosystem has now become so extreme that it is mainly inhabited by halophilic archaea of the <u>Halobacteria</u> family, most of them remaining unclassified, or belonging to the *Halorhabdus* genus (Bodaker et al., 2010; Jacob et al., 2017). The sediment of the Dead Sea is similarly dominated by these phylotypes, and recent studies have argued that the water column communities seed the sedimentary ones, or at least strongly affect the conditions of development of its subsurface biosphere (Ariztegui et al., 2015; Thomas et al., 2015).

However, paleoenvironmental studies using sulfur or carbon isotopes, lipid biomarkers or other biosignatures contained in its sediments have shown that the ancestral Dead Sea hosted more diverse metabolisms. Gypsum minerals for example exhibit specific sulfur isotopic signatures

that are characteristic of sulfate reduction activity (e.g. Torfstein et al., 2005). It is also the case for magnetic minerals such as greigite or titanomagnetite (Ebert et al., 2018; Thomas et al., 2016). The microorganisms responsible for this sulfate reduction however remain unknown. Recently, biomarker evidences also highlighted the recycling of halophilic archaeal membrane by unknown bacterial communities in the deep halite levels of the Dead Sea (Thomas et al., 2018) which constitute a new step in the Dead Sea sedimentary carbon cycle and likely contributes in modifying the pool of sedimentary organic matter of this hypersaline environment. But these bacteria have not been identified and very little information is overall available on the Dead Sea bacterial communities.

As a result, a more precise understanding of the composition of microbial communities of the ancient Dead Sea is critical to fill these gaps and shall help characterizing the paleobiogeochemical conditions leading to the preservation of proxies for paleoclimatic studies. It is also pivotal in understanding how this ecosystem has evolved in the past and how it will react to the increasing pressure on water in the Levantine region (Tahal Group et al., 2011).

Gaining access to these microbial paleocommunities is, however, a complicate matter. The search of lipid biomarkers or mineral biosignatures in bulk sediments lacks in precision and specificity. Additionally, allochtonous organic matter may hide signatures from the Dead Sea paleo-biosphere. We here use the organic fraction preserved in fluid inclusions of halite crystals from salt levels in the deepest core retrieved during the Dead Sea Deep Drilling Project. Fluid inclusions are micrometric cavities that form in the lattice of minerals due to differential precipitation rates. They are particularly numerous in halite, where they trap the fluid from which the crystal precipitated. Providing they remain pristine between their formation and their sampling, they constitute excellent time capsules to capture the chemistry and biology of the water at the time and locus of precipitation. Previous investigations have shown that halite fluid inclusions could preserve DNA of microbial communities as old as 150 ka (Sankaranarayanan et al., 2011). A number of studies also discuss the potential for these fluid inclusions to preserve viable microbes beyond several hundred millions of years (Satterfield et al., 2005; Vreeland et al., 2007, 2000). Although such results are controversial (e.g. Graur and Pupko, 2000) and beyond the scope of this paper, they show that fluid inclusions harbour a large potential for the study of paleo-conditions of life in the Dead Sea during most arid circumstances. Therefore, their study could further illuminate the Dead Sea paleo-biosphere and better constrain its evolution towards current extremes.

2 Material and methods

Core-catcher sediments from DSDDP core 5017-1A (see Neugebauer et al., 2014 for details) have been sampled onshore directly after drilling in December 2010. Specific care was taken to avoid any external or trans-contamination. Right after each drilling shift, cores and core-catchers were brought to the shore and stored in a cooling room (4 °C). Core-catchers were then opened in a specially arranged geomicrobiology lab and sampled for microbiological studies using sterilized clamps and syringes. All samples were stored at -8 °C before further processing. Samples containing halite were selected along the first 200 m of the core. Aliquots were subsampled for microscopic examination under visible and UV light. Pictures of the samples were then taken during core-opening parties at the GFZ of Potsdam.

The recovery of ancient DNA is always an issue in sedimentary samples. It is also the case when working with halite fluid inclusions. As a result, extreme care has been taken to avoid contamination from modern microbes and extraction of DNA and subsequent amplification and cloning of 16S rRNA gene was realized following a protocol tailored specifically for halite fluid inclusions and such little quantity of ancient DNA (Sankaranarayanan et al., 2011). Samples were spiked with known DNA before sterilization of the external walls of the halite crystals with the acid bleach protocol from Sankaranarayanan et al. (2011). Crystals of halite were first soaked in 10N HCl prepared at minimal halite solubility for 15 minutes. They were then transported to a neutralization solution of Na_2CO_3 10 % prepared at halite saturation and to 6 % NaOCl (at halite saturation), for 15 minutes for both. Rinsing in an autoclaved halite saturated brine was performed between each step, in order to avoid reagent carryover, and a final set of 4 washes of 15 minutes each, was done to secure the complete cleansing of the halite crystal walls. After dissolution to 1 M, samples are further desalted using Amicon Centrifugal filters (Ultra-0.5 50 kDa, YM50, Millipore). The filters allow for the trapping of DNA fragments longer than 50 bp only. These fragments were further purified using a minElute PCR Purification Kit (Qiagen). Obtained DNA was quantified using a Nanodrop® ND-1000 Spectrophotometer (Witec AG). To recover sufficient amount of DNA for cloning, an initial PCR done using the universal primers 4F (5'-TCYGGTTGATCCTGCCRG-3') and Univ1492R (5'-CGGTTACCTTGTTACGACTT-3') (Dong et al., 2006) was followed by a nested PCR with 1 µL of PCR using the 3F (5'-TTCCGGTTGATCCTGCCGGA-3') & 9R (5'-CCCGCCATTCCTTTAAGTTTC-3') primers for *Archaea* (Jurgens et al., 1997). For *Bacteria*, we used 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') followed by 341F (5'-

CTCCTACGGGAGGCAGCAGTGGGGAATATTGC-3') and 907R (5'-CCGTCAATTCCTTTRAGTTT-3') (Lane, 1991; Weisburg et al., 1991; Muyzer et al., 1993). DNA fragments were amplified using the following protocole. For *Archaea*, initial denaturation for 5 min at 95°C, 30 cycles of denaturation of 1 min at 95°C, annealation of 1 min at 53°C and elongation for 2 min at 72°C. Final elongation was run for 5 min at 72°C. For *Bacteria*, initial denaturation was run for 2 min at 94°C, and 30 cycles of denaturation at 94°C for 30 sec, annealation for 30 sec at 52°C and elongation for 1 min 30 sec at 72°C. Final elongation was performed at 72°C for 5 min. After purification with High Pure® PCR product Purification Kit (Roche Diagnostic SA), the products were cloned using the TOPO TA Cloning Kit (InvitrogenTM by Life TechnologiesTM) following the manufacturer's instructions. Positive clones were verified by PCR and subsequently prepared for sequencing using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied BioSystems) with primers D4 and R5. Sequencing was performed on an ABIPRISM® 3130xl Genetic Analyzer (Applied BioSystems, Hitachi). All samples yielded positive results (i.e. non-contaminant sequences) except for sample A-82 (206,53 mblf) that never yielded positive clones. Sequences were cleaned, assembled, trimmed to around 550 nucleotides and aligned using CodonCode Aligner© v.3.7.1 (CodonCode Corporation) and MEGA (Kumar et al., 2016). Sequences were checked for chimeras using Bellerophon (Huber et al., 2004) and reference sequences were obtained using the NCBI genbank®. A phylogenetic tree was built through the Weighted Neighbor Joining method (Bruno et al., 2000) under MEGA using 999 bootstrap, and was rooted with *Methanopyrus Kandleri*. Genetic distance with the closest neighbour were also calculated using MEGA. All sequences are available on the Open Science Framework project page https://osf.io/xbj3w/.

3 Results

3.1 Sedimentary analysis

The investigated halite layers could be divided into three different facies (Fig. 1). Sample A-1 is composed of small cumulate crystals. Sample A-2 is a mix of small cumulate crystals and large transparent crystals. Sample A-21 is formed of large transparent halite crystals. Samples A-26, A-42 and A-82 are formed of alternations of small raft crystals and large transparent ones. According to Kiro et al. (2016), small cumulate crystals (rafts) precipitate from the surface of the Dead Sea water column while large transparent ones form in the bottom. As a result,

small cumulate crystals may contain only microorganisms from the photic zone, while large transparent ones may harbor communities from the deep layers, the sediment interface, as well as those that have sedimented to the bottom of the lake (after cell death for example). Microscopic investigation permitted to identify pristine fluid inclusions in all samples (Fig. 2A and 2B). Some of them harbored dark filaments or spots that may correspond to microbial cells. However, the small size of the putative organisms and the limited visibility through halite crystals prevented univocal interpretations (Fig. 2C). Under UV light, autofluoresence could confirm that these elements were of organic origin (Fig. 2D), but did not permit to assert whether they were allochthonous organic matter fragments, or actual microbial cells. In only one sample (A-2 at 1.3 m below lake floor, mblf) a green algal cell from the *Dunaliella* genus has been identified given its higher size, shape and red color (Fig. 2B). This halite interval has been interpreted as precipitated during the anomalously wet winter of 1991-1992 (Kiro et al., 2016) that triggered a bloom of *Dunaliella* (Oren et al., 1995). Hence, it is very likely that this *Dunaliella* cell is a "survivor" of this very bloom.

3.2 Microbiological analysis

In total, 15 different Operational Taxonomic Unit (OTU) - a unit used to classify groups of closely related individuals - (9 from *Archaea* and 6 from *Bacteria*) were retrieved from 5 different halite samples (Table 1). No sequence could be recovered from samples deeper than 87.6 mblf, potentially setting up a limit to the preservation of fossil DNA in the Dead Sea halite fluid inclusions to the beginning of the Holocene (estimated age of the halite level at 87.6 mblf, (Neugebauer et al., 2014).
At 0.24 mblf, one OTU was assigned to the halophilic archaeal family *Halobacteriaceae* (genus *Halorussus,* Minegishi et al., 2011) and one OTU was related to a *Gammaproteobacteria* of the genus *Marinobacter* (Chang et al., 2012). At 1.3 mblf, only one archaeal OTU could be retrieved and was associated to the sulfur reducing archaeal genus *Halodesulfurarchaeum* (Cytryn et al., 2000). At 32.9 mblf, one bacterial OTU was obtained and was related to the *Clostridia Halanaerobium* genus (Podell et al., 2013). The halite sample at 48.12 mblf yielded 9 OTUs, among which 2 were assigned to *Bacteria*. The first one was closely associated to uncultured *Acetothermia* (98 % identity, Emmerich et al., 2012) (Fig. 4) and one assigned to the *Alphaproteobacteria Rhodovibrio* genus (Atanasova et al., 2012). Archaeal OTUs were all related to *Euryarchaeota*. Four OTUs were related to the *Halobacteria* class and 3 of them were associated to the *Haloferacaceae* family (genus *Salinarchaeum* ; Grant et al., 1999; Mesbah et

al., 2007). The other *Halobacteria* related OTUs was assigned to the uncultured MSP41-clade (Rubin et al., 2017). The other *Euryarchaeota* were associated to the *Nanoarchaeaeota* class (Narasingarao et al., 2012). Finally, two bacterial OTUs were obtained from a halite sample at 87.6 mblf, and were assigned to *Acetothermia* (Emmerich et al., 2012) and to the *Desulfovermiculus* genus of *Gammaproteobacteria* (Dillon et al., 2013).

Overall, we obtained a relatively small number of non-contaminant OTUs from halite levels. This is in line with the little amount of material studied, and speculated extremely low number of microbial cells in the paleo-Dead Sea. The oldest DNA sequence dates back to the beginning of the Holocene. They encompass both archaeal and bacterial halophilic representatives that bear new information on the evolution of the extreme microbial ecosystem of the Dead Sea during the current interglacial period.

4 Discussion

Halite is known to deposit in the Dead Sea during periods of low freshwater inputs and intense evaporation. These conditions have dominated along the Holocene, and together with the human activity have contributed to lowering the lake level and increasing its salinity to its minimum current level. During the Holocene, Mg^{2+} concentrations never exceeded the current values but total dissolved salt (TDS) may have reached similar levels (Levy et al., 2018, 2017). We therefore have likely reached today an extreme in terms of microbial life conditions (Oren, 2010).

The Dead Sea life is currently limited to halophilic prokaryotes, which mainly belong to *Archaea* of the *Halobacteria* class. They are the principal inhabitants of the Dead Sea brine (Bodaker et al., 2010; Jacob et al., 2017) and of its shallow sediment (Ionescu et al., 2012; Thomas et al., 2015). Jacob et al. (2017) also identified bacterial sequences using universal prokaryotic primers for the 16S rRNA gene. However, these sequences do not belong to halophilic organisms and some of them are common lab contaminants (Sheik et al., 2018), so must be taken very cautiously, the Dead Sea water being very poor in microbial cells and therefore prone to contamination. Bacterial presence was outlined in several studies, mainly concentrated in the vicinity of freshwater springs (Häusler et al., 2014; Ionescu et al., 2012) where reduced salinities allow their development. Similarly, eukaryotic cells have been outlined after heavy rain episodes, during which they may form red blooms of *Dunaliella* algae

providing sufficient dilution of the epilimnion (Oren, 1993, 1983). Finally, several fungal species have also been isolated although their contribution to the Dead Sea ecosystem is poorly known (Oren and Gunde-Cimerman, 2012).

4.1 Ancient archaeal communities

Our results show that the presence of halophilic archaea has been common during most halite-precipitating periods of the Holocene. The dominance of *Halobacteriaceae* sequences, and more precisely from the genus *Salinarchaeum* (named *Halorhabdus* in the NCBI database) is a common feature of the present Dead Sea community, both in the water column (Bodaker et al., 2010; Jacob et al., 2017) and in the halite sediment (Thomas et al., 2015). It seems that this genus has inhabited the Dead Sea water column for a long time, as similarly affiliated sequences were retrieved in the 48.12 mblf halite, which age is estimated by ^{14}C at 6640 ±45 BP (Kitagawa et al., 2016). *Salinarchaeum*/*Halorhabdus* members have been acknowledged to be highly versatile and may grow in oxic and anoxic environments, preventing any inference on their environmental niche (Werner et al., 2014). *Halobacteria* members from the MSP41-clade, recovered in fluid inclusions at 48.12 mblf, had also been identified through their 16S rRNA gene sequence in the most recent halite deposits of the deep Dead Sea (Thomas et al., 2015), and seem to be also stable inhabitants of the lake waters. Uncultured *Archaea* like *Nanohaloarchaea* have been identified through the sequences of the DS fluid inclusions at 48.12 mblf. Metagenomics data from this class show that they are likely to live in aerobic conditions (Narasingarao et al., 2012) suggesting that they inhabited the Dead Sea water column 6.6 ky ago. No trace of their presence has been recorded in the modern lake but two sequences associated to this lineage were recovered from a microbial mat collected in the vicinity of the Dead Sea, which harbored less extreme conditions than the Dead Sea sediment itself (Thomas et al., 2015). Chlorine and magnesium porewater concentrations suggest that the lake was higher and less hypersaline at that period, potentially allowing these organisms to develop, while a current salinity threshold may have been reached and may prevent their presence. Cultivation of members of the novel class *Nanohaloarchaea*, the second archaeal lineage to host halophilic organisms (Aouad et al., 2018), should help refine the limits for their development (Oren, 2015).

Interestingly, an OTU associated to the *Halodesulfurarchaeum* genus has been identified for the first time in the Dead Sea (in halite at 1.3 mblf). They represent the first trace of identified sulfur reducing archaea in this environment. Their reliance on elemental sulfur, thiosulfate or

DMSO as electron acceptor and on formate or hydrogen as electron donor make them relatively versatile organisms able to compete with heterotrophic or lithototrophic organisms (Sorokin et al., 2017). While these results are only preliminary, they suggest a complex sulfur cycle in the Dead Sea, with stepwise degradation of sulfur species likely involving consortia of archaea and bacteria. This could possibly be reflected in the sulfur isotopes of reduced compounds (iron sulfides, concretions) and of their residual products (Bishop et al., 2013; Torfstein et al., 2005).

4.2 Ancient bacterial communities

The bacterial actor of this sulfur reduction might be linked to *Desulfovermiculus* of the *Desulfohalobiaceae* family (*Gammaproteobacteria*; Table 1), whose 16S rRNA gene sequence was recovered from the halite layer at 87,6 mblf. This is less than one meter above gypsum layers marking the transition between the late Pleistocene stratified Lake Lisan and the early Holocene Dead Sea (Neugebauer et al., 2014; Torfstein et al., 2008), which could thus explain the persistence of this anaerobe in halite fluid inclusions.

Two other halophilic bacterial anaerobes were identified through their fluid inclusion 16S rRNA gene sequences: *Halanaerobium* and *Acethothermia*. The age of the halite from which *Halanaerobium* sequences have been recovered is estimated between 4130 ±40 and 4815 ±35 BP (Kitagawa et al., 2016), a period during which the lake level may have had comparable levels and salinity than today (Levy et al., 2017). Similar organisms were previously identified in a microbial mat in the vicinity of the Dead Sea (Thomas et al., 2015), where salinity was alike the modern Dead Sea. *Acethormia*-related sequences were retrieved from halite layers at 48.12 m and 87.6 m blf. Similar sequences were also retrieved from a microbial mat in the vicinity of the lake and from a layer of authigenic aragonite of the Dead Sea (2.74 mblf; Thomas et al., 2015). They were formerly assigned to the Candidate divison KB1 described from the Kebrit Deep brine in the Red Sea (Eder et al., 1999), but have now been reclassified in the Silva SSU r132 database (Quast et al., 2013) as members of the Acetothermia phylum. However, our sequences cluster within the hypersaline KB1 Division, and out of the thermophilic cluster of *Acetothermia*. Sequences defining the hypersaline KB1 divisions outlined by Nigro et al. (2016), encompass the closest matches of our sequences and were recovered from deep-sea anoxic brines where high Mg^{2+} concentrations -a characteristic feature of the Dead Sea water- were measured (e.g. van der Wielen et al., 2005; Yakimov et al., 2013). We suggest thanks to these new fluid inclusion sequences that these organisms likely persisted in the deep part of the lake, possibly at the water-sediment interface, where they could benefit from sinking organic

carbon. Several studies show that KB1 organisms feed on glycine betaine, an osmotic solute commonly found in halotolerant to halophilic bacteria using the salt-out/low salt-in strategy (Nigro et al., 2016; Yakimov et al., 2013). These organisms hardly survive in the Dead Sea environment, where osmotic equilibration by the salt-in strategy (adopted by KB1 Candidate Division, *Halobacteria* and *Halanaerobiaceae* members) is generally favored (Nigro et al., 2016; Oren, 2008). It is hence possible that KB1 feed on the necromass of halophilic organisms unable to survive in the increasingly harsh conditions of the Dead Sea (Ariztegui et al., 2015; Thomas et al., 2015). The fact that they have not been recovered in the modern lake (Thomas et al., 2015; Jacob et al., 2017) can be explained by increasingly harsh conditions preventing their development or that of halotolerant organisms, which constitute the base of their foodweb. Except for the *Marinobacter* sequence at 0.24 m, all obtained closest matches were retrieved from hypersaline environments (terrestrial or marine). Sequences related to halotolerant species are not expected to develop in the halite-precipitating Dead Sea water column, salinity conditions presumably being too harsh for them. They have been more likely transported to the lake before being trapped in the fluid inclusion. Finally, the recovery of a halophilic phototrophic sequence of *Rhodovibrio*, rare in the modern Dead Sea, underlines the potential of fluid inclusion extractions for trapping surficial water column communities to the sediment.

4.3 Evolution of the Dead Sea ecosytem - An outlook

The fluid inclusions data provide new information on the diversity and complexity of the Dead Sea microbial ecosystems, in the sediment and the water column. They suggest previously unconsidered actors and point towards new direction regarding the study of paleobiogeochemical cycles of this environment.

The modern Dead Sea ecosystem is one of the harshest on Earth in terms of salinity and divalent cation concentrations. During the Holocene, the lake level has fluctuated and rarely reached as extreme values as today. We show here that although hypersaline conditions have prevailed during the Holocene, short term dilution or lowering of the salinity have allowed the develoment of bacterial members, and poorly known archaeal organisms. It is the case of KB1/Acetothermia, MSP41 or Halanaerobium. They may have persisted in more favorable environments such as those provided by freshwater springs near the Dead Sea shores (Häusler et al., 2014; Ionescu et al., 2012), which today harbour similar phylotypes (Thomas et al., 2015). Other organisms (*Halodesulfurarchaeum*, *Nanohaloarchaea* and *Desulfohalobius*) are identified in the Dead Sea realm for the first time. It is possible that increasingly harsh

conditions may have led to their disappearance. It is also possible that the low coverage of certain 16S rRNA gene sequencing (Thomas et al., 2015), poor coverage of halophilic sequences database (Thomas et al., 2014) or specificity of the used primers (Bodaker et al., 2010; Jacob et al., 2017) can contribute to their non-recovery in previous studies.

Important similarities are shared between the deep halite-hosted subsurface biosphere of the Dead Sea (be it of Holocene or older age) and ancient DNA obtained from halite fluid inclusions. Given the extreme attention devoted in avoiding contamination from modern communities, we suggest that these similarities advocate for a seeding of the subsurface biosphere by the lake water column ecosystem. Although data remain scarce to unequivocally prove this pattern, the extreme conditions inherent to this ecosystem is likely to select for organisms within the water column, first for their ability to withstand osmotic pressure. In addition to being extreme halophiles, the dominant organisms (namely *Halorhabdus*/ *Salinarchaeum*) of the sediment and the water column have the potential for developing in both oxic and anoxic environments. This versatility may very well be a favored trait in the Dead Sea subsurface ecosystem. Local environmental conditions, are therefore likely to be reflected in both the paleo and modern sedimentary communities.

While a unique ecosytem has managed to adapt to the extreme conditions of the Dead Sea, it seems that the pressure on this environment will keep on growing, and little is known on the way microbes will react. Their adaptative ability is immense and already overcomes numerous limitations imposed by the medium. Our current work provide avenues for the study of these limitations and associated adaptations, and shows that although extreme, such organisms are indeed affected by environmental changes.

Data availability

The data are available on the Open Science Framework project page https://osf.io/xbj3w/.

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Fig. 1: Investigated halite layers from the ICDP Dead Sea Deep Drilling Project cores. Fluid inclusions from the Dead Sea core 5017-A. (A) Small rafts precipitated from the shallow depth of the modern Dead Sea water column (core A-1). (B) Large transparent halite crystals mixed with mud from core A-2. (C) Large transparent halite crystals (core A-21). (D) Alternations of small white halite crystals and large transparent ones from core A-26. (E) Alternations of small white halite crystals and large transparent ones from core A-42. (F) Mix of small white halite crystals and large transparent ones from core A-82. Sample widths are 7 cm.
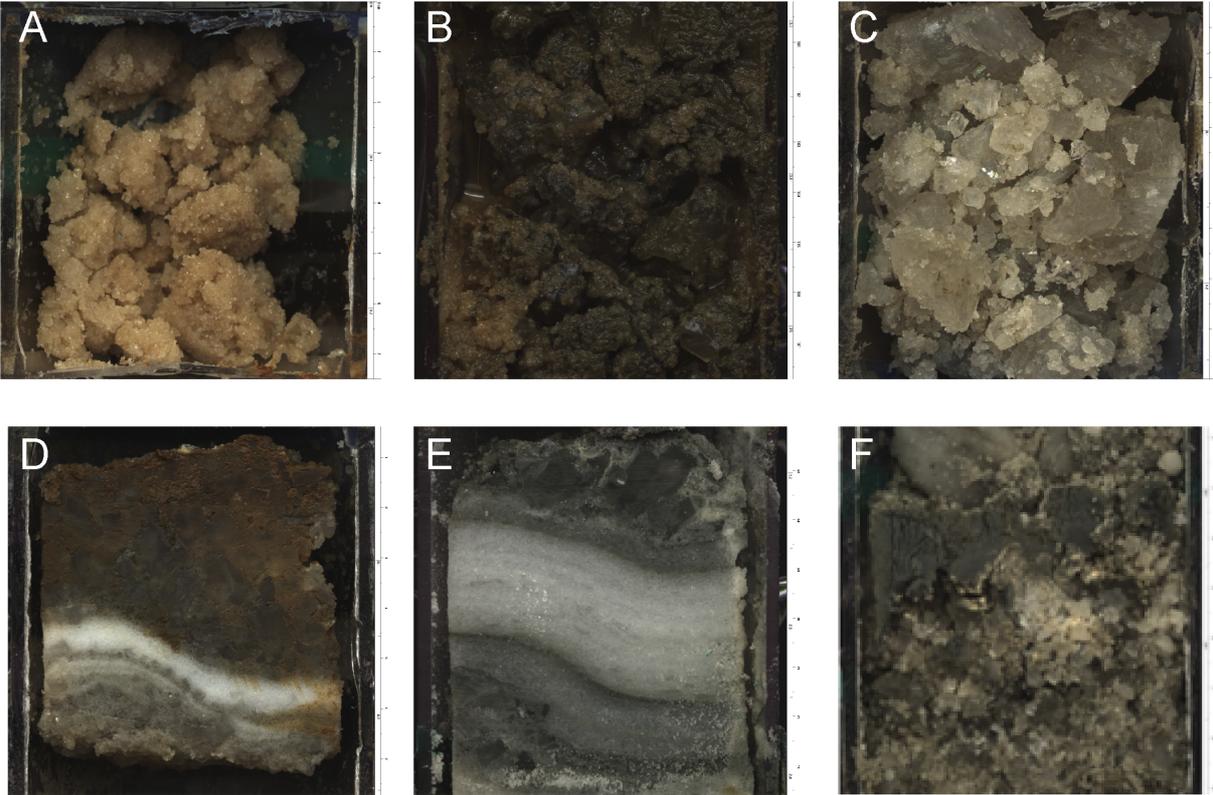
Fig. 2: Fluid inclusions from the Dead Sea core 5017-A. (A) Example of primary cubic fluid inclusions from which DNA extraction was attempted. (B) Halophilic algae *Dunaliella* enclosed in a fluid inclusion of halite sample in core A-2 (1.3 mblf). Organic matter is not often noticeable under normal light (C) but can be clearly see through its auto-fluorescence under UV light (D). Scale bars are 50 µm except for (B).
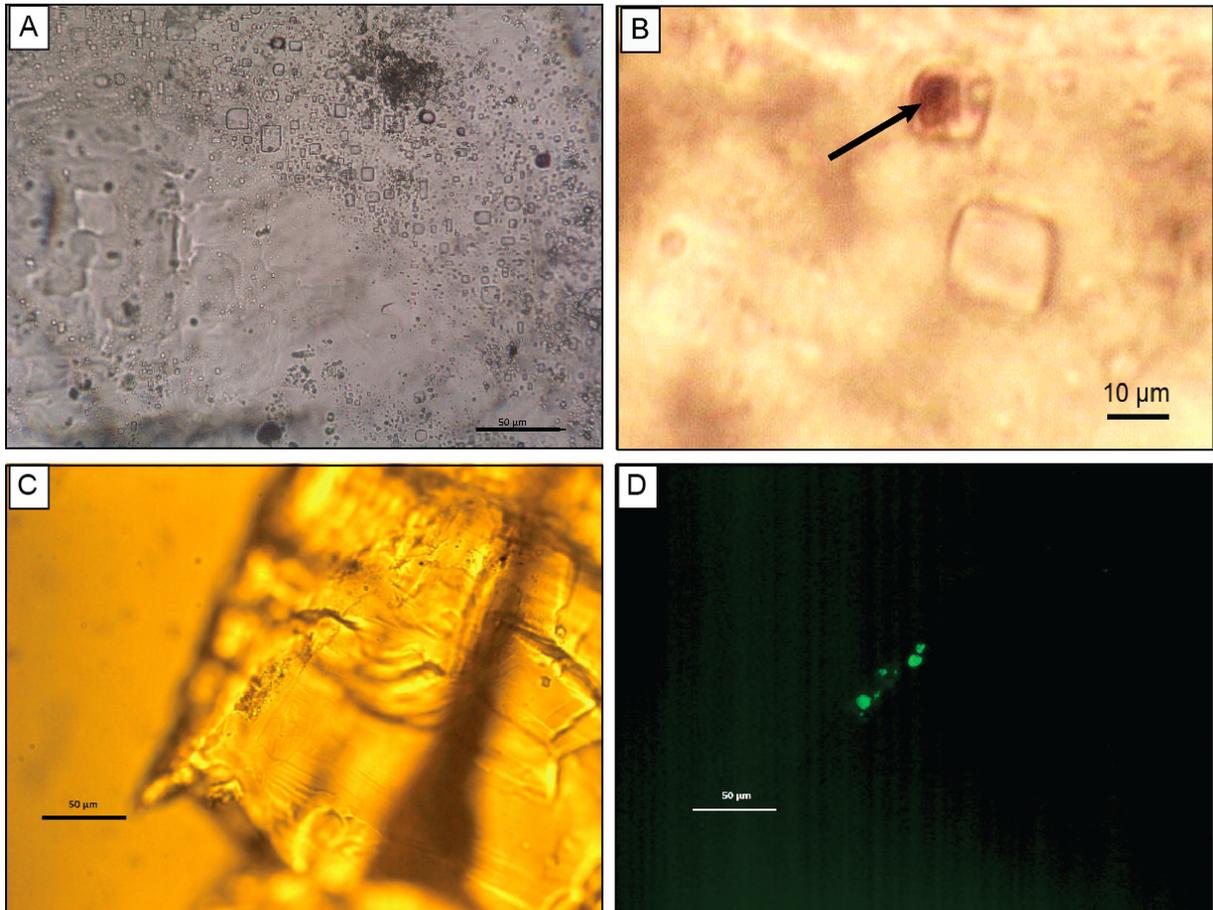
Fig. 3 : Neighbour joining tree of archaeal and bacterial 16S rRNA gene sequences retrieved from halite fluid inclusions from the Dead Sea Deep Drilling Project, together with their associated closest matches (NCBI).
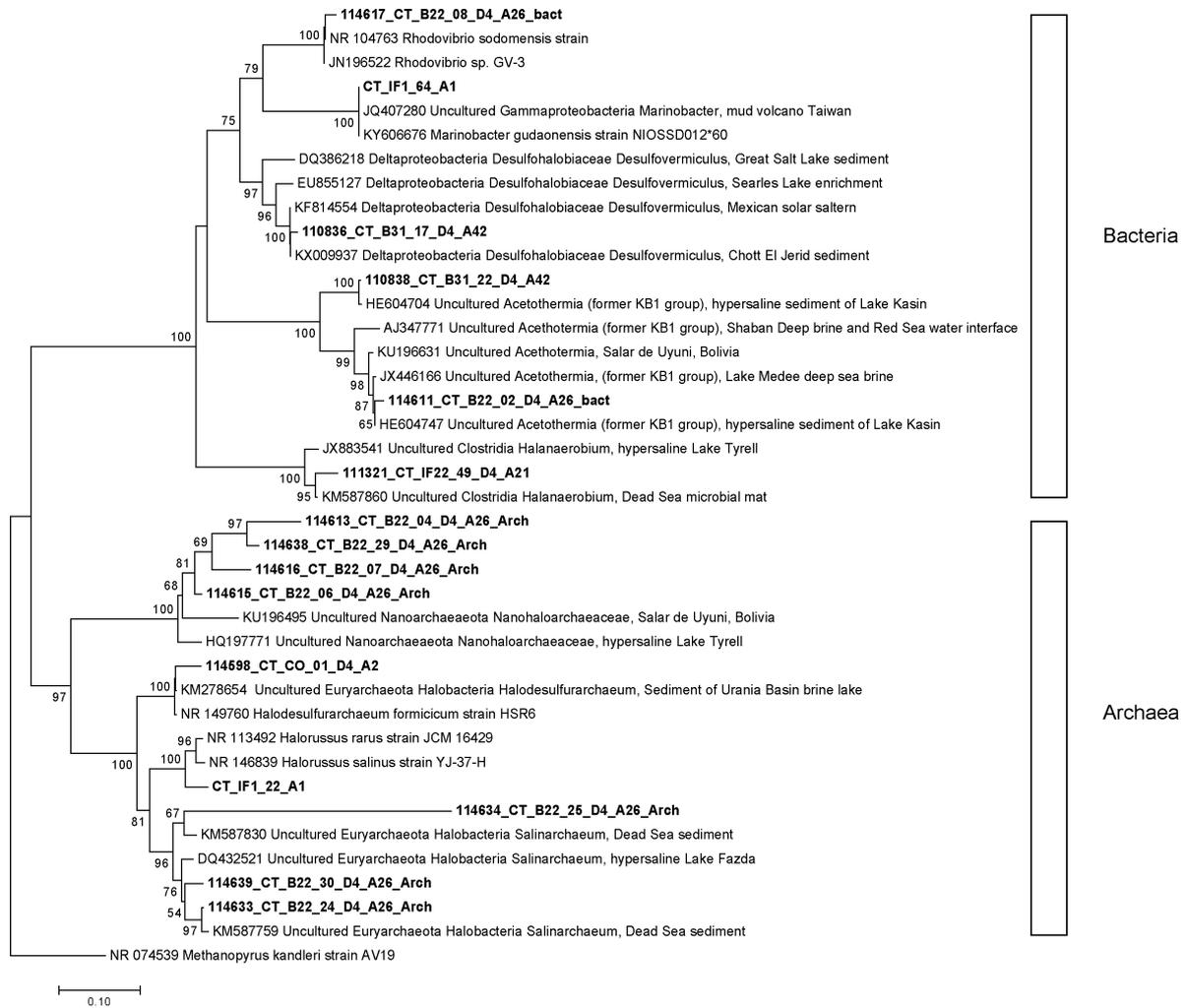
Table 1. Sequences recovered from fluid inclusions of the Dead Sea core samples and information from the closest matches

Analyzed fluid inclusions										closest match			
Core	Depth (m)	Age	Domain	Phylum	Class	Order	Family	Genus	OTU	Identity (%)	Environment	Oxygen	Reference
A-1	0,24	2010	Archaea	Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	Halorussus	A01_01	95	Cultured	yes	Minegishi et al., 2012
A-1	0,24	2010	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Marinobacter	A01_02	99	Mud volcano sediment	no	Chang et al., 2012
A-2	1,3	1991-1992	Archaea	Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	Halodesulfurarchaeum	A02_01	96	Hypersaline stratified lake	no	Cytryn et al., 2000
A-21	32,9	4130 ±40 to 4815 ±35 BP	Bacteria	Firmicutes	Clostridia	Clostridiales	Halanaerobiaceae	Halanaerobium	A21_01	90	Hypersaline lake water	yes	Podell et al., 2013
A-26	48,12		Archaea	Euryarchaeota	Halobacteria	Halobacteriales	Haloferacaceae	Salinarchaeum	A26_01	98	Hypersaline lake water	yes	Mesbah et al., 2007
A-26	48,12		Archaea	Euryarchaeota	Halobacteria	Halobacteriales	Haloferacaceae	Salinarchaeum	A26_02	95	Hypersaline lake water	yes	Mesbah et al., 2007
A-26	48,12		Archaea	Euryarchaeota	Halobacteria	Halobacteriales	Haloferacaceae	Salinarchaeum	A26_03	86	Alkaline saltern water	yes	Grant et al., 1999
A-26	48,12		Archaea	Euryarchaeota	Nanohaloarchaea	Nanohaloarchaeales	Nanohaloarchaeaceae	Uncultured	A26_04	90	Hypersaline crystallizer pond water	yes	Narasingarao et al., 2012
A-26	48,12	6640 ±45 BP	Archaea	Euryarchaeota	Nanohaloarchaea	Nanohaloarchaeales	Nanohaloarchaeaceae	Uncultured	A26_05	93	Hypersaline crystallizer pond water	yes	Narasingarao et al., 2012
A-26	48,12		Archaea	Euryarchaeota	Halobacteria	Halobacteriales	MSP41-clade	Uncultured	A26_06	88	Salar water	yes	Rubin et al., 2017
A-26	48,12		Archaea	Euryarchaeota	Nanohaloarchaea	Nanohaloarchaeales	Nanohaloarchaeaceae		A26_07	86	Hypersaline crystallizer pond water	yes	Narasingarao et al., 2012
A-26	48,12		Bacteria	Acetothermia	Uncultured	-	-	-	A26_08	98	Hypersaline lake sediment	no	Emmerich et al., 2012
A-26	48,12		Bacteria	Proteobacteria	Alphaproteobacteria	Rhodovibrionales	Fodinicurvataceae	Rhodovibrio	A26_09	99	Solar saltern water	yes	Atanasova et al., 2012
A-42	87,6	11100 ±110 to 12500 ±250 BP	Bacteria	Acetothermia	Uncultured	-	-	-	A42_01	99	Hypersaline lake sediment	no	Emmerich et al., 2012
A-42	87,6	11100 ±110 to 12500 ±250 BP	Bacteria	Proteobacteria	Gammaproteobacteria	Desulfovibrionales	Desulfohalobiaceae	Desulfovermiculus	A42_02	96	Solar saltern water	yes	Dillon et al., 2013