

Recycling of archaeal biomass as a new strategy for extreme life in Dead Sea deep sediments

Camille Thomas^{1*}, Vincent Grossi^{2*}, Ingrid Antheaume², and Daniel Ariztegui¹

¹Department of Earth Sciences, University of Geneva, Rue des Maraichers 13, 1205 Geneva, Switzerland

²Laboratoire de Géologie de Lyon, Université de Lyon, Centre National de la Recherche Scientifique (CNRS), 69622 Villeurbanne, France

ABSTRACT

Archaea and Bacteria that inhabit the deep subsurface (known as the deep biosphere) play a prevalent role in the recycling of sedimentary organic carbon. In such environments, this process can occur over millions of years and requires microbial communities to cope with extremely limited sources of energy. Because of this scarcity, metabolic processes come at a high energetic cost, but the ways heterotrophic microbial communities develop to minimize energy expenses for a maximized yield remain unclear. Here, we report molecular biomarker evidence for the recycling of archaeal cell wall constituents in extreme evaporitic facies of Dead Sea deep sediments. Wax esters derived from the recombination of hydrolyzed products of archaeal membrane lipids were observed in gypsum and/or halite sedimentary deposits down to 243 m below the lake floor, implying the reutilization of archaeal necromass possibly by deep subsurface bacteria. By recycling the building blocks of putatively better-adapted archaea, heterotrophic bacteria may build up intracellular carbon stocks and mitigate osmotic stress in this energy-deprived environment. This mechanism illustrates a new pathway of carbon transformation in the subsurface and demonstrates how life can be maintained in extreme environments characterized by long-term isolation and minimal energetic resources.

INTRODUCTION

In extreme environments, microbial metabolic processes that lower the energetic cost of maintaining life are favored (Hoehler and Jørgensen, 2013). Such settings are characterized by low growth rates (Lomstein et al., 2012), and most energy is diverted to maintenance functions (van Bodegom, 2007), such as osmotic equilibration, oxygen stress defense, motility, and more sustainable metabolic pathways. These selective conditions for life promote the dominance of prokaryotes and generally favor Archaea relative to Bacteria (Hoehler and Jørgensen, 2013). This is mostly due to the reduced membrane permeability of Archaea, which requires less maintenance energy with respect to bacterial membranes (Valentine, 2007). This advantage is particularly striking in environments characterized by high osmotic stress such as hypersaline environments. There, bacteria may use alternative strategies that allow competition with the putatively better-adapted archaea, for example, by recycling available organic molecules as osmotic solutes

(Oren, 1999a). The intracellular accumulation of ambient organic carbon is a common way of economizing energy in harsh environments. For example, under stressed growth conditions, some Bacteria species are known to accumulate intracellular lipid droplets (Alvarez et al., 1997; Wältermann and Steinbüchel, 2005) in the form of polyhydroxyalkanoates, triglycerides, or wax esters (WEs). However, the presence of such mechanisms in the deep biosphere has not yet been documented, suggesting that they may not be sufficiently effective in these low-energy settings for bacteria to survive.

The Dead Sea is the most saline lake on Earth and has deposited evaporitic minerals since the early Quaternary (Stein, 2001). As a result, its subsurface environment constitutes one of the most extreme ecosystems on the planet. The extreme chemistry of the water allows only for the survival of halophilic archaea in the water column and recent halite sediments (Bodaker et al., 2010; Thomas et al., 2015). Bacteria have rarely been observed in the most extreme sedimentary facies of the Dead Sea (halite or gypsum), suggesting that these harsh conditions limit their growth (Thomas et al., 2015). To further investigate the

composition of the microbial community and its potential metabolic strategies to survive the most arid periods of the late Quaternary of the Dead Sea basin, we characterized the lipid biomarker composition of deep hypersaline halite and gypsum sediments. The good lipid preservation in these extreme horizons gives clues to metabolic pathways that allow for the survival of a thus-far-unrecognized deep biosphere.

METHODS

The material used in this study originated from the International Continental Scientific Drilling Program (ICDP) Dead Sea Deep Drilling Project (DSDDP) core 5017–1A retrieved from the center of the Dead Sea in winter 2010–2011 (Fig. 1). Sediments were sampled on site from core catchers using sterile tools and kept in the freezer at -20°C until further processing. The main characteristics of samples analyzed for lipid biomarkers are given in Table DR1 in the GSA Data Repository¹. Samples were freeze-dried, ground, and extracted using multiple sonication cycles (methanol 2 \times , methanol/dichloromethane [DCM] [1:1 v/v, 2 \times], and DCM 3 \times). Elemental sulfur was removed with activated copper. Lipid extracts were filtered and separated using a deactivated silica gel column (5% H₂O) into five fractions of increasing polarity (hexane/DCM [9:1 v/v], hexane/DCM [1:1 v/v], DCM, ethyl acetate, and methanol). Fractions 3 and 4 were silylated with pyridine/bis(trimethylsilyl) trifluoroacetamide (BSTFA) at 2:1 (v/v). Fraction 5 was trans-esterified by overnight incubation with 0.5 mL of toluene and 2 mL of 2% H₂SO₄ in methanol at 60 °C. Following the addition of NaCl (5%), the organic phase was extracted with hexane:DCM (4:1 v/v, 3 \times), washed with NaHCO₃ (2%), and dried with sodium sulfate before silylation.

All fractions were analyzed by gas chromatography mass spectrometry (GC-MS) on an HP 6890 Series Plus gas chromatograph equipped

*E-mails: camille.thomas@unige.ch; vincent.grossi@univ-lyon1.fr

¹GSA Data Repository item 2019179, supplementary Figures DR1 and DR2 and Tables DR1 and DR2, is available online at <http://www.geosociety.org/datarepository/2019/>, or on request from editing@geosociety.org.

CITATION: Thomas, C., Grossi, V., Antheaume, I., and Ariztegui, D., 2019, Recycling of archaeal biomass as a new strategy for extreme life in Dead Sea deep sediments: *Geology*, v. 47, p. 479–482, <https://doi.org/10.1130/G45801.1>

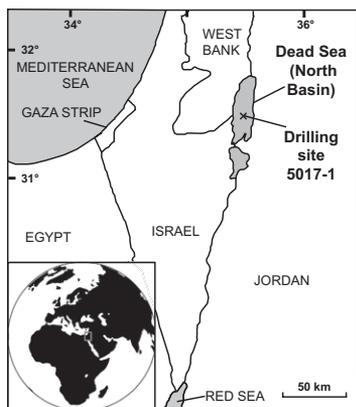


Figure 1. Localization of Dead Sea cores. Material used in this study originated from International Continental Scientific Drilling Program (ICDP) Dead Sea Deep Drilling Project (DSDDP) drilling site 5017-1.

with a cool on-column injector, and coupled to an Agilent 5975C (VL MSD) mass spectrometer. The GC was equipped with an HP5 column (30 m × 0.25 mm × 0.25 μm, RESTEK). Samples were injected at 60 °C (held for 30 s), and the oven temperature was increased to 130 °C at a rate of 20 °C/min, then to 250 °C (5 °C/min) and 300 °C (3 °C/min), and finally held isothermal for 45 min. Stepwise dilution of external standards allowed quantification. Isoprenoid fatty acids are less polar than linear acids and partly eluted in fraction F4. A ratio based on specific bacterial fatty acids was calculated using the sum of methyl-branched C₁₅ and C₁₇ fatty acids over the sum of linear C₁₅ and C₁₇ fatty acids. Changes in this ratio indicate a shift in bacterial community.

Compound-specific carbon isotope (δ¹³C) analyses were done using a HP7890B GC coupled to an Isoprime visION isotope ratio mass spectrometer via a GC-5 combustion interface operating at 870 °C. The GC was equipped with a BPX5 column (30 m × 0.25 mm × 0.10 μm, SGE Analytical Science) and a cool on-column injector, and the oven temperature was programmed as for GC-MS analyses. The B4 standard mixture (Arndt Schimmelmann, Indiana University, USA) was used to externally calibrate the δ¹³C values, and the known BSTFA derivatizing agent δ¹³C value was corrected for alcohols and fatty acids. After samples were decalcified, total organic carbon (TOC) was measured, and data were normalized to a standard sediment (IVA Analysentechnik, Germany) containing 9.15% of carbon, measured every 10 samples.

RESULTS AND DISCUSSION

We found significant amounts of isoprenoid wax esters (WEs; up to 0.2 μg g⁻¹ TOC) in halite and gypsum samples retrieved between 90 and 250 m below lake floor (mblf; Fig. 2; Table DR1). WEs are an important type of energy-storage molecule that can be produced by Eukarya and Bacteria, particularly under conditions of stress, but they have not been reported in the archaeal domain

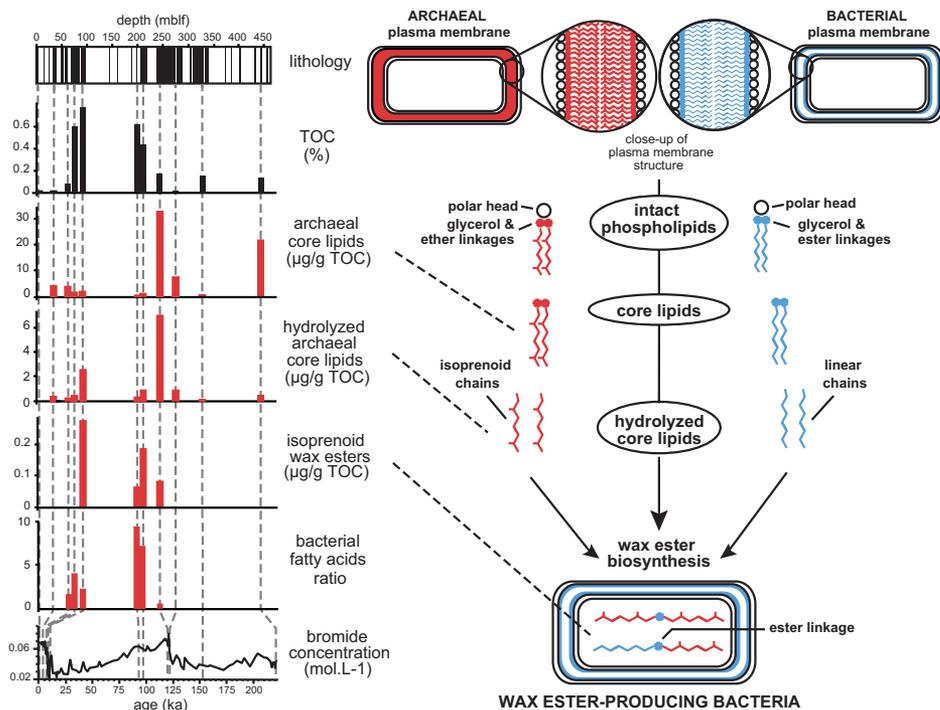


Figure 2. Occurrence of wax esters (WEs) and other lipid biomarkers in Dead Sea core 5017-1 (gypsum and halite intervals are shown in black; dashed lines indicate position of samples analyzed). “Hydrolyzed core lipids” refer to degradation products of archaeol and extended archaeol, which are presented as alkyl chains. TOC—total organic carbon; mblf—meters below lake floor. Bacterial fatty acids ratio (sum of methyl-branched C₁₅ and C₁₇ fatty acids) indicates presence of bacteria potentially involved in WE production. Sampled layers are compared to bromide concentration in sediment pore waters (used as a proxy for lake-water dilution/concentration; Levy et al., 2017) and fitted to ¹⁴C ages (Kitagawa et al., 2016) and U/Th ages (Torstein et al., 2015). Formation of WE building blocks is schematized on right-hand side of figure. Red items indicate an archaeal origin; blue items indicate a bacterial origin.

so far (Wang et al., 2019). WEs are formed by condensation of fatty acids and alcohols available in the environment and provide easily accessible (intracellular) sources of carbon (Waltermann and Steinbüchel, 2005). The major WE detected was phytanyl phytanate (3,7,11,15-tetramethylhexadecyl-3,7,11,15-tetramethylhexadecanoate; iC₂₀-iC₂₀; Fig. 3), which was accompanied by a series of other isoprenoid WEs composed of a C₂₀ or C₂₅ isoprenoid alcohol esterified to a linear, methyl-branched or isoprenoid acyl chain (Fig. 3A). The isoprenoid WEs were accompanied by significant amounts of membrane core lipids of halophilic archaea—archaeol (2,3-*O*-diphytanyl-*sn*-glycerol) and extended archaeol (2,3-*O*-phytanyl-*O*-sesterterpanyl-*sn*-glycerol; Kates, 1997; Dawson et al., 2012)—along with several of their hydrolyzed and eventually oxidized products (Figs. 2 and 3B; Table DR2). Lipids of halophilic archaea are composed of C₂₀ and C₂₅ isoprenoid alkyl chains, which allow for better control of membrane permeability under strong osmotic stress compared to bacterial fatty acid membranes (Valentine, 2007; Koga, 2012). Archaeol was the most abundant isoprenoid alcohol in most of the investigated sediment samples (Figs. 2 and 3). Extended archaeol, a membrane lipid more specific to halophilic archaea of the Halobacteria class (Dawson et al., 2012) that dominate the Dead Sea halite and gypsum

sediments (Thomas et al., 2015), was also found to be enriched in halite and gypsum facies. In most of the sedimentary intervals where WEs occurred, archaeol and extended archaeol were found in lower abundance than in the other halite/gypsum samples (Fig. 2). All WE-containing intervals also contained hydrolyzed core lipids and some oxidized counterparts (Fig. 2), which consisted of C₂₀ and C₂₅ isoprenoid alcohols and acids (phytanol, phytanic acid, and C₂₅ homologues), and of isoprenoid C₂₀ and C₂₅ monoalkyl glycerol monoethers (Fig. 3B; Table DR2). The report of WEs with isoprenoid C₂₅ carbon chains (iC₂₀-iC₂₅ and iC₂₅-iC₂₀) in environmental samples is unprecedented and attests to the incorporation of archaeal core lipid subunits into isoprenoid WEs. The formation of isoprenoid WEs has been previously demonstrated during growth of bacteria on free isoprenoid compounds such as phytane, phytol, or squalene (Rontani et al., 1999, 2003; Silva et al., 2007). The recycling of phytol derivatives into mixed isoprenoid and non-isoprenoid WEs has also been reported in desiccated mats from modern evaporative alkaline lakes (Finkelstein et al., 2010). However, phytane, phytol, and squalene were not observed in the investigated samples from the Dead Sea. As a result, the WEs detected in the Dead Sea halite/gypsum samples mostly consist of lipid subunits derived from the cell walls of archaea, the dominant organisms in the

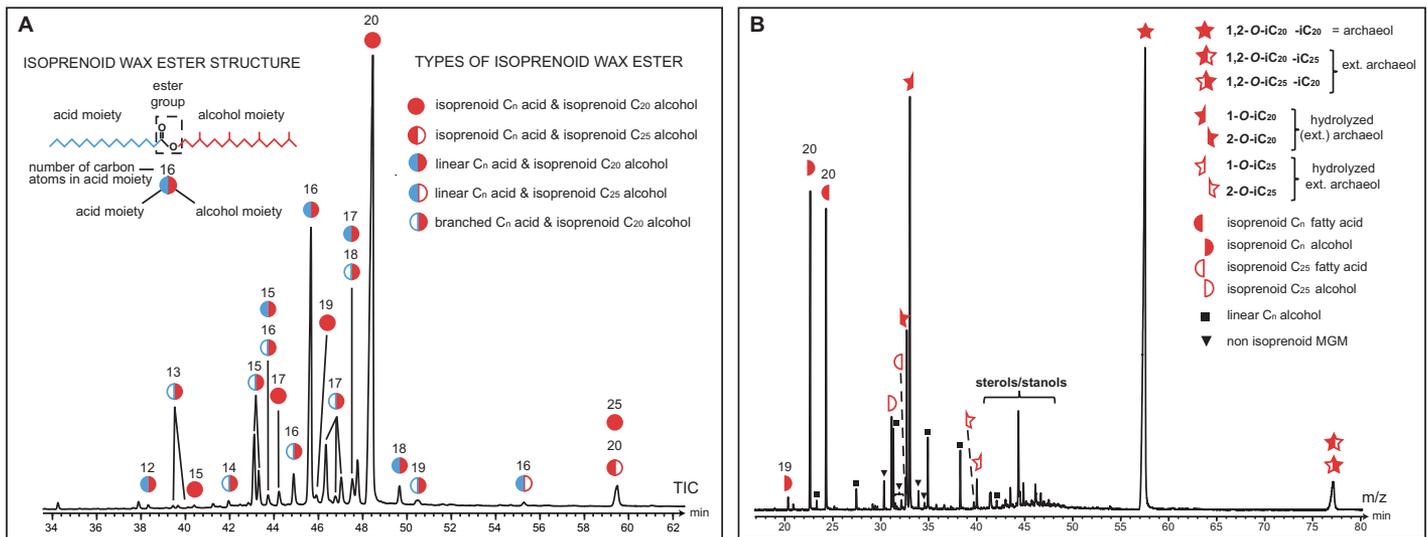


Figure 3. Characteristic chromatograms of isoprenoid wax esters (WEs) and related building blocks. A: Total ion chromatogram (TIC) of WE fraction (fraction 2) from gypsum sample at 91.04 m below lake floor (mblf). **B:** Extracted ion chromatogram (m/z 103+133+159+205+218) of a polar lipid fraction (fraction 4) from gypsum sample at 243.22 mblf. These intervals were selected because they show highest concentrations of isoprenoid WEs and hydrolyzed core lipids, respectively. MGM—monoalkyl glycerol monoethers. Red symbols indicate an archaeal origin; blue symbols indicate a bacterial/eukaryal origin. Numbers above symbols shown in B correspond to number of carbon atoms in chain.

Dead Sea (Bodaker et al., 2010; Thomas et al., 2015). This recycling was further confirmed by compound-specific stable carbon isotope analyses (Fig. DR1), showing that the $\delta^{13}\text{C}$ values of WEs agree with those of their building blocks (fatty acids and alcohols).

Current knowledge suggests that the ability to form and accumulate WEs has only arisen in the Bacteria and Eukarya Domains (Garay et al., 2014; Wang et al., 2019). Eukaryotic life in the Dead Sea has been constrained to humid intervals during glacial periods and occasional blooms triggered by high precipitation during interglacial stages (Oren et al., 1995). Hence, isoprenoid WEs present in the most arid intervals of the Dead Sea sediment (halite/gypsum) cannot have originated from eukaryotes. So far, archaea have never been shown to produce WEs. Given the prevalence of archaea in the Dead Sea environment, and the fact that ubiquitous groups of archaea have been shown to possess the genetic machinery for fatty acid synthesis (Iverson et al., 2012; Villanueva et al., 2017), an archaeal origin of the isoprenoid WEs cannot be completely ruled out. However, recent bioinformatics analysis of presently available archaeal genomes (including some hyperhalophiles) has failed to identify homologues of genes coding for a wax ester synthase (Wang et al., 2019). Abiotic esterification has also been suggested to occur during the transformation of sedimentary lipids (Becker, 2015). However, the activity of extracellular enzymes is likely to be inhibited in the hypersaline Dead Sea sediment (Frankenberger and Bingham, 1982; Grant, 2004), and, in the present case, putative abiotic reactions would have yielded a much wider diversity of ester structures (including eukaryotic sterols). The predominance of specific bacterial C_{15} and C_{17} fatty acids (Perry et al., 1979) in WE-containing sedimentary intervals (Fig. 2) instead

suggests a bacterial origin of the WEs. Recent work on fluid inclusions allowed the retrieval of bacterial sequences in halite-dominated layers of the deep Dead Sea sediment (Thomas and Ariztegui, 2019). Our data would therefore support the development of bacteria upon archaeal necromass in the extreme environment of the Dead Sea subsurface.

The concentration of intracellular WEs in bacteria has been shown to arise particularly in situations of nutrient starvation, especially when nitrogen is limiting (Ishige et al., 2003). Additionally, the recycling of archaeal biomass by archaea themselves was suggested to minimize the energy cost of life in nutrient- and carbon-limited environments (Takano et al., 2010). Here, we propose that the recycling of archaeal necromass into storage lipids like WEs constitutes a way to save and store energy in the nutrient- and energy-demanding hypersaline environment of the Dead Sea. The presence of WEs with

mixed linear and isoprenoid acyl chains alongside the predominant isoprenoid WEs indicates that the WE-forming microbial population also recycled bacterial and/or eukaryal fatty acids, in addition to archaeal lipids (Fig. 4). This evidence suggests that the subsurface biosphere creates easily accessible carbon stocks from necromass, in a lake with rare allochthonous inputs and primary production (Oren, 1999b).

The accumulation of WEs has previously been reported in episodically desiccated microbial mats in hypersaline environments (Finkstein et al., 2010), and this was suggested to allow for better microbial cell survival during periods of desiccation, due to the release of H_2O during esterification. The Dead Sea sediments that bear WEs originate from the deepest part of the lake (i.e., 297.5 m below lake level), where neither traces of desiccation (Neugebauer et al., 2016) nor microbial mats have been reported. These sedimentary levels correspond, however, to

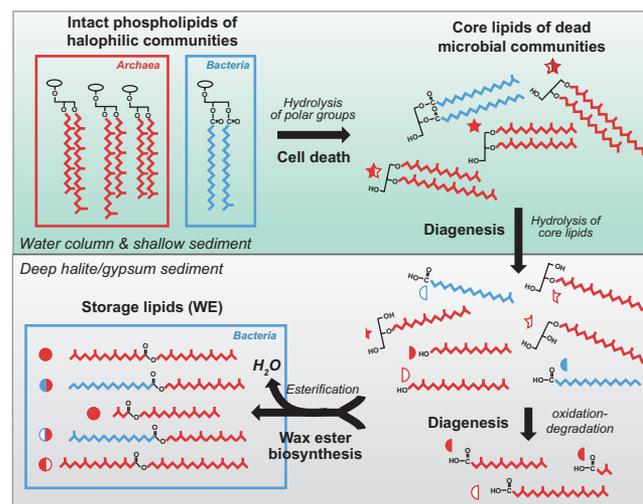


Figure 4. Schematic pathway for isoprenoid wax ester (WE) formation by recycling of membrane lipids in Dead Sea sediments. Intact phospholipids from archaeal and bacterial/eukaryal communities are hydrolyzed and eventually oxidized, and some of these degradation products are transformed into WEs by bacterial communities in deep halite/gypsum sediment horizons. Symbols refer to compounds identified in Figure 3. Red moieties have an archaeal origin; blue moieties have a bacterial/eukaryal origin.

periods when salinity was the highest in the deep brine, as supported by the bromide concentration curve (Fig. 2), a reliable salinity proxy for the Dead Sea water column during the Quaternary (Levy et al., 2017). The WE-bearing levels generally correspond to the highest concentrations of this element (Fig. 2; Table DR1), which are observed during the driest climatic intervals (marine isotopic stages 5E, early 5A, and the 2/1 transition). Water availability is a major issue, not only in dry environments, but also in hypersaline settings due to high salt concentrations inducing osmotic stress. By recombining hydrolyzed moieties of core lipids originating from the buried archaeal necromass, the WE-forming bacterial population may therefore have created accessible water molecules that favored its survival.

Our results illustrate the high adaptability of the subsurface biosphere and its ability to use varied strategies for energy production and preservation under adverse conditions. By studying an environment that pushes life to its limits, we catch a glimpse of the processes that fuel life in the deep subsurface, and we advance our understanding of the deep sedimentary carbon cycle.

ACKNOWLEDGMENTS

We thank the scientific and technical teams of the Dead Sea Deep Drilling Project (DSDDP, www.icdp-online.org), and A. Vinçon-Laugier, A. Vuillemin, and M. Makou. Sponsorship from the International Continental Scientific Drilling Program and funding by the Swiss National Science foundation (projects 200021–132529 and 200020–149221/1 to Ariztegui) and the French National Research Agency (grant ANR-12-BSV7–0003 to Grossi) are acknowledged. We are grateful to Yuki Weber and an anonymous reviewer for their very constructive comments on an earlier version of the manuscript.

REFERENCES CITED

Alvarez, H.M., Pucci, O.H., and Steinbüchel, A., 1997, Lipid storage compounds in marine bacteria: Applied Microbiology and Biotechnology, v. 47, p. 132–139, <https://doi.org/10.1007/s002530050901>.

Becker, K.W., 2015, Biogeochemical Significance and Biomarker Potential of Novel Glycerolipids and Respiratory Quinones in the Marine Environment [Ph.D. thesis]: Bremen, Germany, Universität Bremen, 306 p.

Bodaker, I., et al., 2010, Comparative community genomics in the Dead Sea: An increasingly extreme environment: The ISME Journal, v. 4, p. 399–407, <https://doi.org/10.1038/ismej.2009.141>.

Dawson, K.S., Freeman, K.H., and Macalady, J.L., 2012, Molecular characterization of core lipids from halophilic archaea grown under different salinity conditions: Organic Geochemistry, v. 48, p. 1–8, <https://doi.org/10.1016/j.orggeochem.2012.04.003>.

Finkelstein, D.B., Brassell, S.C., and Pratt, L.M., 2010, Microbial biosynthesis of wax esters during desiccation: Adaptation for colonization of the earliest terrestrial environments?: Geology, v. 38, p. 247–250, <https://doi.org/10.1130/G30398.1>.

Frankenberger, W.T., Jr., and Bingham, F.T., 1982, Influence of salinity on soil enzyme activities: Soil Science Society of America Journal, v. 46, p. 1173–1177, <https://doi.org/10.2136/sssaj1982.03615995004600060011x>.

Garay, L.A., Boundy-Mills, K.L., and German, J.B., 2014, Accumulation of high-value lipids in single-cell microorganisms: A mechanistic approach and future perspectives: Journal of Agricultural and Food Chemistry, v. 62, p. 2709–2727, <https://doi.org/10.1021/jf4042134>.

Grant, W.D., 2004, Life at low water activity: Philosophical Transactions of the Royal Society of London, ser. B, Biological Sciences, v. 359, p. 1249–1267, discussion 1266–1267, <https://doi.org/10.1098/rstb.2004.1502>.

Hoehler, T.M., and Jørgensen, B.B., 2013, Microbial life under extreme energy limitation: Nature Reviews–Microbiology, v. 11, p. 83–94, <https://doi.org/10.1038/nrmicro2939>.

Ishige, T., Tani, A., Sakai, Y., and Kato, N., 2003, Wax ester production by bacteria: Current Opinion in Microbiology, v. 6, p. 244–250, [https://doi.org/10.1016/S1369-5274\(03\)00053-5](https://doi.org/10.1016/S1369-5274(03)00053-5).

Iverson, V., Morris, R.M., Frazar, C.D., Berthiaume, C.T., Morales, R.L., and Armburst, E.V., 2012, Untangling genomes from metagenomes: Revealing an uncultured class of marine euryarchaeota: Science, v. 335, p. 587–590, <https://doi.org/10.1126/science.1212665>.

Kates, M., 1997, Diether and tetraether phospholipids and glycolipids as molecular markers for Archaeobacteria (Archaea): Molecular Markers in Environmental Geochemistry, v. 671, p. 35–48, <https://doi.org/10.1021/bk-1997-0671.ch003>.

Kitagawa, H., Stein, M., Goldstein, S.L., Nakamura, T., and Lazar, B., and DSDDP Scientific Party, 2016, Radiocarbon chronology of the DSDDP core at the deepest floor of the Dead Sea: Radiocarbon, v. 59, p. 1–12, <https://doi.org/10.1017/RDC.2016.120>.

Koga, Y., 2012, Thermal adaptation of the archaeal and bacterial lipid membranes: Archaea (Vancouver, B.C.), v. 2012, p. 789652, <https://doi.org/10.1155/2012/789652>.

Levy, E.J., Stein, M., Lazar, B., Gavieli, I., Yechieli, Y., and Sivan, O., 2017, Pore fluids in Dead Sea sediment core reveal linear response of lake chemistry to global climate changes: Geology, v. 45, p. 3–6, <https://doi.org/10.1130/G38685.1>.

Lomstein, B.A., Langerhuus, A.T., D’Hondt, S., Jørgensen, B.B., and Spivack, A.J., 2012, Endospore abundance, microbial growth and necromass turnover in deep sub-seafloor sediment: Nature, v. 484, p. 101–104, <https://doi.org/10.1038/nature10905>.

Neugebauer, I., Schwab, M.J., Waldmann, N.D., Tjallingii, R., Frank, U., Hadzhiivanova, E., Naumann, R., Taha, N., Agnon, A., Enzel, Y., and Brauer, A., 2016, Hydroclimatic variability in the Levant during the early last glacial (~117–75 ka) derived from micro-facies analyses of deep Dead Sea sediments: Climate of the Past, v. 12, p. 75–90, <https://doi.org/10.5194/cp-12-75-2016>.

Oren, A., 1999a, Bioenergetic aspects of halophilism: Microbiology and Molecular Biology Reviews, v. 63, p. 334–348.

Oren, A., 1999b, Microbiological studies in the Dead Sea: Future challenges toward the understanding of life at the limit of salt concentrations: Hydrobiologia, v. 405, p. 1–9, <https://doi.org/10.1023/A:1003879932328>.

Oren, A., Gurevich, P., Anati, D., Barkan, E., and Luz, B., 1995, A bloom of *Dunaliella parva* in the Dead Sea in 1992: Biological and biogeochemical aspects: Hydrobiologia, v. 3, p. 173–185, <https://doi.org/10.1007/BF00019283>.

Perry, G.J., Volkman, J., and Johns, R., 1979, Fatty acids of bacterial origin in contemporary marine sediments: Geochimica et Cosmochimica Acta, v. 43, p. 1715–1725, [https://doi.org/10.1016/0016-7037\(79\)90020-6](https://doi.org/10.1016/0016-7037(79)90020-6).

Rontani, J.F., Bonin, P.C., and Volkman, J.K., 1999, Biodegradation of free phytol by bacterial communities isolated from marine sediments under aerobic and denitrifying conditions: Applied and Environmental Microbiology, v. 65, p. 5484–5492.

Rontani, J.F., Mouzdahir, A., Michotey, V., Caumette, P., and Bonin, P., 2003, Production of a polyunsaturated isoprenoid wax ester during aerobic metabolism of squalene by *Marinobacter squalenivorans* sp. nov.: Applied and Environmental Microbiology, v. 69, p. 4167–4176, <https://doi.org/10.1128/AEM.69.7.4167-4176.2003>.

Silva, R.A., Grossi, V., and Alvarez, H.M., 2007, Biodegradation of phytane (2,6,10,14-tetramethylhexadecane) and accumulation of related isoprenoid wax esters by *Mycobacterium ratisbonense* strain SD4 under nitrogen-starved conditions: FEMS Microbiology Letters, v. 272, p. 220–228, <https://doi.org/10.1111/j.1574-6968.2007.00770.x>.

Stein, M., 2001, The sedimentary and geochemical record of Neogene–Quaternary water bodies in the Dead Sea Basin—Inferences for the regional paleoclimatic history: Journal of Paleolimnology, v. 26, p. 271–282, <https://doi.org/10.1023/A:101752928186>.

Takano, Y., Chikaraishi, Y., Ogawa, N.O., Nomaki, H., Morono, Y., Inagaki, F., Kitazato, H., Hinrichs, K.-U., and Ohkouchi, N., 2010, Sedimentary membrane lipids recycled by deep-sea benthic archaea: Nature Geoscience, v. 3, p. 858–861, <https://doi.org/10.1038/ngeo983>.

Thomas, C., and Ariztegui, D., 2019, Fluid inclusions from the deep Dead Sea sediment provide new insights on Holocene extreme microbial life: Quaternary Science Reviews (in press).

Thomas, C., Ionescu, D., and Ariztegui, D., 2015, Impact of paleoclimate on the distribution of microbial communities in the subsurface sediment of the Dead Sea: Geobiology, v. 13, p. 546–561, <https://doi.org/10.1111/gbi.12151>.

Torfstein, A., Goldstein, S.L., Kushnir, Y., Enzel, Y., Haug, G., and Stein, M., 2015, Dead Sea drawdown and monsoonal impacts in the Levant during the last interglacial: Earth and Planetary Science Letters, v. 412, p. 235–244, <https://doi.org/10.1016/j.epsl.2014.12.013>.

Valentine, D.L., 2007, Adaptations to energy stress dictate the ecology and evolution of the Archaea: Nature Reviews–Microbiology, v. 5, p. 316–323, <https://doi.org/10.1038/nrmicro1619>.

van Bodegom, P., 2007, Microbial maintenance: A critical review on its quantification: Microbial Ecology, v. 53, p. 513–523, <https://doi.org/10.1007/s00248-006-9049-5>.

Villanueva, L., Schouten, S., and Damsté, J.S.S., 2017, Phylogenomic analysis of lipid biosynthetic genes of Archaea shed light on the ‘lipid divide’: Environmental Microbiology, v. 19, p. 54–69, <https://doi.org/10.1111/1462-2920.13361>.

Wältermann, M., and Steinbüchel, A., 2005, Neutral lipid bodies in prokaryotes: Recent insights into structure, formation, and relationship to eukaryotic lipid depots: Journal of Bacteriology, v. 187, p. 3607–3619, <https://doi.org/10.1128/JB.187.11.3607-3619.2005>.

Wang, L., Liu, Q., Wu, X., Huang, Y., Wise, M.J., Liu, Z., Wang, W., Hu, J., and Wang, C., 2019, Bioinformatics analysis of metabolism pathways of archaeal energy reserves: Scientific Reports, v. 9, p. 1–12, <https://doi.org/10.1038/s41598-018-37768-0>.

Printed in USA