Title

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

Thomas[1]* Camille, Grossi[2]* Vincent, Antheaume[2] Ingrid, Ariztegui[1] Daniel

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

[2] Laboratoire de Géologie de Lyon, Université de Lyon, CNRS, UCBL, ENSL, Villeurbanne, , France

* Corresponding authors (Camille.thomas@unige.ch; Vincent.grossi@univ-lyon1.fr)

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 extreme environment, this process can occur over millions of years (Lomstein et al., 2012) and requires microbial communities to cope with 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 by Bacteria in extreme evaporitic facies of Dead Sea deep sediments. Wax esters (WE) derived from the recombination of hydrolyzed products of archaeal membrane lipids were observed in gypsum and/or halite sedimentary deposits down to 243 meters below the lake floor (mblf), implying the reutilization of archaeal necromass by deep subsurface Bacteria. By recycling the building blocks of putatively better adapted Archaea, heterotrophic Bacteria build up intracellular carbon stocks and gain access to free water in this deprived environment. This mechanism illustrates a new pathway of carbon transformation in the subsurface and reveals how life is maintained in extreme environments characterized by long-term isolation and minimal energetic resources.

Keywords: deep biosphere, archaeal membrane recycling, bacterial storage lipids, isoprenoid wax esters, Dead Sea hypersaline sediments.

Significance

The deep subsurface constitutes one of the frontiers of life exploration. While, the micro-organisms inhabiting this extreme environment play a fundamental role in the Earth carbon

cycle, the life modes of the deep biosphere are poorly understood. In general, members of the domain Archaea are better adapted to such extreme environments, where they survive at hardly measurable metabolic rates. We here present an original strategy developed by Bacteria to survive in the deep hypersaline sediments underlying the Dead Sea, which is based on the recycling of dead archaeal biomass. These observations support a new micro-organismal survival strategy that advances our knowledge of life adaptation, inter-domain interactions and carbon cycling in the deep biosphere.

Introduction

In extreme environments, any metabolic process that significantly lowers the energetic cost of life is favored (1). In such settings, growth rates decrease (2) and most energy is diverted to maintenance functions (3) such as osmotic equilibration, O_2 stress defense, motility, or shifts to sustainable metabolic pathways. These selective conditions for life promote the dominance of prokaryotes and generally favor Archaea relative to Bacteria (1). This is mostly due to the reduced membrane permeability of Archaea, which requires less maintenance energy with respect to bacterial membranes (4). 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 (5). The intracellular accumulation of available organic carbon is a common way of economizing energy in harsh environments. For example, under stressed growth conditions, some Bacteria are known to accumulate intracellular lipid droplets (6, 7) in the form of polyhydroxyalkanoates, triglycerides or wax esters. However, the occurrence of such mechanisms in the deep biosphere has not yet been documented suggesting that they may not be satisfactory for Bacteria to survive in these low energy settings.

The Dead Sea is the most saline lake on Earth and has deposited evaporitic minerals since the early Quaternary (8). As a result, its subsurface environment constitutes one of the most extreme ecosystem on the planet. High salinity (38.5 $g.L^{-1}$) and divalent cation concentrations (Ca^{2+}= 508 mM and Mg^{2+}= 1952 mM) of the water allow only for the survival of halophilic Archaea in the water column and recent halite sediment (9–11). Archaea seem to be favored by their osmotic equilibration strategy, which is based on the pumping of cation through their cell membrane, when other organisms (Eukarya and most Bacteria) generally need to integrate or synthesize small osmotic solutes intracellularly to equilibrate with the external medium (12). This so-called "salt-in" strategy is energetically cheaper that the synthesis of osmotic solutes, and hence allows to save energy for other metabolic purpose. While some Bacteria harbor similar salt-in osmotic equilibration strategy (13), their presence has rarely been observed in the most extreme sedimentary facies of the Dead Sea (halite or gypsum), which suggests that the extreme conditions of these environments still limit their development (11).

By analyzing the composition and molecular structure of lipid biomarkers of the deep hypersaline sediment underlying the Dead Sea, we here provide unprecedented evidence for an original strategy developed by Bacteria to survive in this harsh environment. The characterization of isoprenoid wax esters in deep halite and gypsum sediments demonstrates the recycling of archaeal membrane by Bacteria (the only prokaryotic domain able to synthesize esters), and supports a new bacterial strategy to develop and survive in the deep biosphere.

Results and discussion

The lipid composition of different sedimentary facies obtained through the Dead Sea Deep Drilling Project showed the presence of significant amounts of isoprenoid WE (up to 0.2 $\mu g.g^{-1}$ TOC) in halite and gypsum samples retrieved between 90 and 250 mblf (Fig. 1 and Table S1). Wax esters are a type of energy storage molecules that can be formed and accumulated by

109 Eukarya and Bacteria, particularly under conditions of stress, but that have not been reported
110 in the archaeal domain (14). The formation of WE arises from the condensation of linear or
111 isoprenoid alkyl chains available in the environment and provides easily accessible
112 (intracellular) sources of carbon (7). The WE detected in the Dead Sea halite/gypsum samples
113 mostly consist of lipid subunits derived from the cell walls of Archaea, the dominant organisms
114 in the lake (10, 15). The major WE detected was phytanyl phytanate (3,7,11,15-
115 tetramethylhexadecyl-3,7,11,15-tetramethylhexadecanoate; iC_{20}-iC_{20}, Fig. 2) which was
116 accompanied by series of other isoprenoid WE composed of a C_{20} or C_{25} isoprenoid alcohol
117 esterified to a linear, methyl-branched or isoprenoid acyl chain (Fig. 2a). The isoprenoid WE
118 were systematically observed together with significant amounts of membrane core lipids of
119 halophilic Archaea - archaeol (2,3-*O*-diphytanyl-*sn*-glycerol) and extended archaeol (2,3-*O*-
120 phytanyl-*O*-sesterterpanyl-*sn*-glycerol) (16, 17) - along with several of their hydrolyzed (and
121 eventually oxidized) products (Figs 1 and 2b; Table S2). Lipids of halophilic Archaea are
122 specifically composed of C_{20} and C_{25} isoprenoid alkyl chains, which allow for better control of
123 membrane permeability under strong osmotic conditions compared to bacterial fatty acid
124 membranes (4, 18). Archaeol was the most abundant isoprenoid alcohol in most of the
125 investigated sediment samples (Figs. 1 and 2). Extended archaeol, a membrane lipid more
126 specific to halophilic Archaea of the Halobacteria class (16) that dominate the Dead Sea halite
127 and gypsum sediments (11), was also found enriched in halite and gypsum facies. In most of
128 the sedimentary intervals where WE occurred, archaeol and extended archaeol were found in
129 lower abundance than in the other halite/gypsum samples (Fig. 1). Conversely, the hydrolyzed
130 products of these archaeal membrane lipids were preferentially observed in the WE-rich
131 intervals (Fig. 1), and mainly consisted of C_{20} and C_{25} isoprenoid alcohols and acids (phytanol,
132 phytanic acid and C_{25} homologues), and of *sn*-1 and *sn*-2 isoprenoid C_{20} and C_{25} monoalkyl
133 glycerols (Fig 2b and supplementary data). The report of WE with isoprenoid C_{25} carbon chains

(iC_{20}-iC_{25} and iC_{25}-iC_{20}) in natural samples is unprecedented and testifies of the use of core lipid subunits from halophilic Archaea as a source for the isoprenoid alkyl and acyl chains present in the WE. The formation of isoprenoid WE has been previously demonstrated during growth of Bacteria on free isoprenoid compounds such as phytane, phytol or squalene (19–21). However, such isoprenoid compounds were not observed in the investigated samples from the Dead Sea. Together, our results clearly indicate the reutilization of archaeal membrane lipids to form isoprenoid WE.

Further evidence for such a process was obtained from compound specific stable carbon isotope analyses. The ^{13}C composition of degradation products of archaeol and extended archaeol measured in a halite sample from 206.53 mblf was similar to those of the co-occurring isoprenoid WE (Fig. 3). For instance, the $\delta^{13}C$ of C_{20} and C_{25} isoprenoid alcohols (-24.03 ‰ ±0.37 and -24.70 ‰ ±0.62, respectively) were very close to the values measured for WE composed of these isoprenoid moieties (-24.51 ‰ ±0.18 for iC_{20}-iC_{20} and -24.70 ‰ ±0.78 for the mixture iC_{20}-iC_{25} + iC_{25}-iC_{20}). An intermediate degradation product of archaeol and extended archaeol, the C_{20} monoalkyl glycerol (1-*O*-iC_{20}), also showed a similar $\delta^{13}C$ value (-25.40 ‰ ±0.95). These isotope composition similarities strongly support an archaeal origin of the isoprenoid moieties incorporated in WE. The ^{13}C composition of WE composed of a mixture of isoprenoid and linear (or methyl-branched) acyl chains (i.e. WE nC_{16}-iC_{20}) were also in agreement with an archaeal origin of the isoprenoid moieties (Fig. 3). The slightly heavier ^{13}C composition of archaeol compared to archaeol hydrolysis products and isoprenoid WE (Fig. 3) can be explained by the presence of different archaeal populations that may have developed asynchronously in the same sediment interval.

The characterization of isoprenoid WE has been shown to represent a useful tool for deciphering the metabolic pathways employed by microorganisms for the degradation of isoprenoid substrates (19–21). The condensation of isoprenoid compounds with each other or

159 with their degradation products to form isoprenoid WE indeed allows the preservation of
160 transient metabolites, which would not necessarily be preserved in their free form. Structural
161 analysis of the different WE present in the Dead Sea halite samples allowed us to decipher the
162 metabolic pathways involved in the degradation of archaeal core lipids (Fig. S2). Following
163 hydrolysis of the ether linkages present in archaeol and extended-archaeol, a portion of the
164 released isoprenoid C_{20} and C_{25} alcohol moieties was oxidized to the corresponding isoprenoid
165 fatty acids, providing the building blocks for biosynthesis of iC_{20}-iC_{20}, iC_{25}-iC_{20} and iC_{20}-iC_{25}
166 WE. Part of the isoprenoid fatty acids was further degraded via successive α-oxidation, β-
167 oxidation and β-decarboxymethylation sequences (19, 21), providing the acyl chains for shorter
168 isoprenoid WE (Fig. 2a). The identification of iC_5-iC_{20} WE in some samples demonstrates the
169 almost complete degradation of the isoprenoid alkyl chains released from the hydrolysis of
170 archaeol and extended archaeol, providing carbon and energy to the deep biosphere.
171
172 Current knowledge suggests that the ability to form and accumulate WE has only arisen in
173 bacterial and eukaryal domains (14). Eukaryotic life in the Dead Sea has been constrained to
174 humid intervals during glacial periods and occasional blooms triggered by high precipitation
175 during interglacial stages (11, 22). Hence, isoprenoid WE present in the most arid intervals of
176 the Dead Sea sediment (halite/gypsum) cannot originate from eukaryotes. The predominance
177 of specific bacterial methyl-branched C_{15} and C_{17} fatty acids in the sedimentary intervals where
178 WE were produced (Fig. 1) instead indicates a bacterial origin of the latter (23). Methyl-
179 branched odd-carbon-number fatty acids are common in sulfate-reducing Bacteria (24). Our
180 data therefore support the development of Bacteria upon archaeal necromass in the extreme
181 environment of the Dead Sea subsurface.
182 The concentration of intracellular WE in Bacteria has been shown to arise particularly in
183 situations of nutrient starvation, especially when nitrogen is limiting (25). Additionally, the

184 recycling of archaeal biomass by Archaea themselves was suggested to minimize the energy
185 cost of life in nutrient- and carbon-limited environments (26). Here, we propose that the
186 bacterial recycling of archaeal necromass into storage lipids like WE constitutes a way to save
187 and store energy in the nutrient- and energy-demanding hypersaline environment of the Dead
188 Sea. The presence of WE with mixed linear and isoprenoid chains alongside dominant
189 isoprenoid WE indicates that the WE-forming bacterial population also recycled bacterial
190 and/or eukaryal lipids, in addition to those of Archaea (Figs. 1, 2 and 4). As a result, Bacteria
191 create easily accessible carbon stocks from necromass, in a lake with very little allochthonous
192 inputs and rare primary production (27). The accumulation of WE has been previously
193 demonstrated in temporarily emerged microbial mats in hypersaline environments (28), and
194 was suggested to allow for better microbial cell survival during periods of desiccation, due to
195 the release of H_2O during esterification. The Dead Sea sediments that bear WE originate from
196 the deepest part of the lake, where no trace of desiccation has been observed (29). These
197 sedimentary levels correspond, however, to periods where salinity was the highest in the deep
198 brine, as supported by the bromide concentration curve (Fig. 1). The latter is interpreted as a
199 reliable salinity proxy for the Dead Sea water column during the Quaternary (30). The WE-
200 bearing levels generally correspond to the highest concentrations of this conservative element
201 (Fig. 1; Table S1), which are observed during the driest climatic intervals (Marine Isotopic
202 Stages 5E, early 5A and the 2/1 transition). Water availability is a major issue not only in dry
203 environments, but also in hypersaline settings due to high salt concentrations. By recombining
204 hydrolyzed moieties of core lipids originating from the buried archaeal necromass, the WE-
205 forming bacterial population therefore creates accessible water molecules that favor its survival.
206
207 The conditions under which the early Holocene or Pleistocene halite were deposited are
208 expected to be similar to the present-day environment that allows halite precipitation from the

Dead Sea water column (30). If such conditions were favorable to the formation of WE, the occurrence of these lipids would also be expected in the surficial halite sediment. Since this is not the case, WE biosynthesis likely occurred deeper in the sedimentary column. The formation of WE has been documented for aerobic microorganisms and environments (19, 31, 32), but their biosynthesis by anaerobic microorganisms has also been recognized (33, 34). Thus, the unique occurrence of isoprenoid WE in the deep halite/gypsum deposits (dated from late Marine Isotopic Stage 5 to the early Holocene) suggests their production within the anoxic sediment, in agreement with the occurrence of C-odd fatty acids specific of anaerobic Bacteria (Fig. 1). Wax esters are generally poorly preserved in the sedimentary record (31), although some of these compounds have been reported in ca. 40 ka-old lacustrine sediments (35). The age of the Dead Sea sediments containing the isoprenoid WE has been estimated to be between 120 and 11.4 ka (Table S1) based on U-Th in aragonite (36) and ^{14}C dating (37). This supports that under certain conditions, wax esters can be preserved under much longer periods of time.

The present investigation illustrates the high adaptability of Bacteria and their 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 add a new loop to the deep sedimentary carbon cycle.

Methods

During the drilling campaign, sediments were sampled from core catchers using sterile tools, and kept in the freezer until further processing. The main characteristics of samples analyzed for lipid biomarkers are given in Table S1. Samples were freeze-dried, ground and extracted using multiple sonication cycles (methanol x2, methanol/dichloromethane (1:1, x2) and dichloromethane x3). Elemental sulfur was removed with activated copper. Lipid extracts were

234 filtered and separated using a deactivated silica gel column (5% H_2O) into five fractions of
235 increasing polarity. Fraction F1 was eluted with hexane/dichloromethane (9:1), fraction F2 with
236 hexane/dichloromethane (1:1), fraction F3 with dichloromethane, fraction F4 with ethyl acetate
237 and fraction F5 with methanol. Fractions F3 and F4 were silylated with pyridine/BSTFA 2:1
238 (v/v). Fraction F5 was trans-esterified by incubating at 60°C overnight with toluene (0.5 ml)
239 and 2% H_2SO_4 in methanol (2 ml). NaCl 5% was then added and the organic phase was
240 extracted three times with hexane:dichloromethane (4:1), washed with $NaHCO_3$ (2%) and dried
241 with sodium sulfate. The dry extract was silylated with pyridine/BSTFA 2:1 (v/v) before
242 analysis by gas chromatography-mass spectrometry (GC-MS). GC-MS analyses were
243 performed on a HP 6890 Series Plus gas chromatograph equipped with a cool on-column
244 injector and coupled to an Agilent 5975C (VL MSD) mass spectrometer. The samples were
245 injected at 60°C and the oven temperature was programmed as follows: 60 °C isotherm for 30
246 sec, 20 °C /min to 130 °C, 5 °C /min to 250 °C and 3 °C/min to 300 °C (45 minutes isotherm).
247 Compound identification was based on interpretation of mass spectral characteristics and
248 comparison of these spectra and retention times with those of authentic standards or literature
249 data. Quantification was realized using stepwise dilution of external alcohol, ester and alkane
250 standards. Peak areas were integrated manually using the ChemStation software. Isoprenoid
251 fatty acids are less polar than linear acids and partly eluted in the alcohol fraction.
252 Compound-specific carbon isotope ($\delta^{13}C$) analyzes were performed using a HP7890B gas
253 chromatograph (GC) coupled to an Isoprime visION isotope ratio mass spectrometer via a GC-
254 5 combustion interface operating at 870°C. The GC was equipped with a BPX5 column (30 m
255 × 0.25 mm × 0.10 µm) and a cool on-column injector, with helium as the carrier gas (1 ml/min
256 flow rate). The samples were injected at 60°C and the oven temperature was ramped to 130°C
257 at 20°C/min, then to 300°C (held for 40 min) at 4°C/min. The samples were analyzed in
258 duplicate or triplicate and the measured $\delta^{13}C$ values were corrected first for instrument

deviation using the Indiana University B4 standard mixture and then for the BSTFA derivatizing agent (for alcohols and fatty acids).

Bulk organic C analyses were performed using an Elementar Vario Micro Cube coupled to a Thermal Conductivity Detector. Between 1 and 8 mg of bulk sediment were weighed into tin capsules which were introduced into the combustion furnace (950°C) with an excess of oxygen. Copper oxide was used as oxidation catalyst and He as carrier gas. Reduction of N_xO_y to N_2 and removal of excess O_2 was achieved with reduced copper at 550°C. Water was removed with a phosphorous pentoxide chemical trap. N_2 and CO_2 were separated with a purge and trap desorption column. Data were normalized to a working standard of IVA sediment containing 9.15% of C, which was measured every ten samples.

Calculation of the bacterial fatty acid ratio (Fig. 1) was performed using the sum of branched methyl C_{15} and C_{17} fatty acids over the sum of linear C_{15} and C_{17} fatty acids.

The authors declare no conflict of interest.

The data that support the findings of this study are available from the corresponding authors.

Acknowledgments

Sampling was realized in collaboration with Aurèle Vuillemin and with the staff at the International Continental Drilling Project core repository at GFZ-Potsdam. We wish to thank the scientific and technical teams of the Dead Sea Deep Drilling Project (www.icdp-online.org). Arnauld Vinçon-Laugier is acknowledged for his assistance in the organic geochemistry lab. We also thank Matthew Makou for help in editing the manuscript and constructive comments. The material was collected thanks to the sponsorship of ICDP, and funded by the Swiss National Science foundation (projects 200021-132529 and 200020-149221/1). Organic

Geochemical analyses were funded by the French National Research Agency/Agence Nationale de la Recherche (grant ANR-12-BSV7-0003 to VG).

Author Contributions

CT, VG and DA planned the project and designed the analytical approach. CT and DA contributed to the sampling. CT and IA realized the organic geochemistry analyses. CT, VG and DA interpreted the results and wrote the manuscript. DA and VG secured the funding necessary for the study. All authors provided editorial comments on the manuscript.

Reference

1. Hoehler TM, Jørgensen BB (2013) Microbial life under extreme energy limitation. *Nat Rev Microbiol* 11(2):83–94.
2. Lomstein BA, Langerhuus AT, D'Hondt S, Jørgensen BB, Spivack AJ (2012) Endospore abundance, microbial growth and necromass turnover in deep sub-seafloor sediment. *Nature* 484(7392):101–104.
3. Van Bodegom P (2007) Microbial maintenance: A critical review on its quantification. *Microb Ecol* 53(4):513–523.
4. Valentine DL (2007) Adaptations to energy stress dictate the ecology and evolution of the Archaea. *Nat Rev Microbiol* 3(April):316–323.
5. Oren A (1999) Bioenergetic Aspects of Halophilism. *Microbiol Mol Biol Rev* 63(2):334–348.
6. Alvarez HM, Pucci OH, Steinbüchel A (1997) Lipid storage compounds in marine bacteria. *Appl Microbiol Biotechnol* 47(2):132–139.
7. Wältermann M, Steinbüchel A (2005) Neutral Lipid Bodies in Prokaryotes : Recent Insights into Structure, Formation, and Relationship to Eukaryotic Lipid Depots. *J*

Bacteriol 187(11):3607–3619.

8. Stein M (2001) The sedimentary and geochemical record of Neogene- Quaternary water bodies in the Dead Sea Basin – inferences for the regional paleoclimatic history *. *J Paleolimnol* 26:271–282.

9. Ionescu D, et al. (2012) Microbial and chemical characterization of underwater fresh water springs in the Dead Sea. *PLoS One* 7(6):e38319.

10. Bodaker I, et al. (2010) Comparative community genomics in the Dead Sea: an increasingly extreme environment. *ISME J* 4(3):399–407.

11. Thomas C, Ionescu D, Ariztegui D (2015) Impact of paleoclimate on the distribution of microbial communities in the subsurface sediment of the Dead Sea. *Geobiology* 13(6):546–561.

12. Oren A (2010) Thermodynamic limits to microbial life at high salt concentrations. *Environ Microbiol* 13:1908–1923.

13. Oren A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. *Saline Systems* 4:2.

14. Murphy DJ (2012) The dynamic roles of intracellular lipid droplets: From archaea to mammals. *Protoplasma* 249(3):541–585.

15. Rhodes ME, Oren A, House CH (2012) Dynamics and persistence of Dead Sea microbial populations as shown by high-throughput sequencing of rRNA. *Appl Environ Microbiol* 78(7):2489–92.

16. Dawson KS, Freeman KH, Macalady JL (2012) Molecular characterization of core lipids from halophilic archaea grown under different salinity conditions. *Org Geochem* 48:1–8.

17. Kates M (1997) Diether and tetraether phospholipids and glycolipids as molecular markers for Archaebacteria (Archaea). *Mol Markers Environ Geochemistry* 671:35–48.

18. Koga Y (2012) Thermal adaptation of the archaeal and bacterial lipid membranes. *Archaea* 2012.

19. Silva R a, Grossi V, Alvarez HM (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 Microbiol Lett* 272(2):220–8.

20. Rontani JF, Mouzdahir A, Michotey V, Caumette P, Bonin P (2003) Production of a polyunsaturated isoprenoid wax ester during aerobic metabolism of squalene by Marinobacter squalenivorans sp. nov. *Appl Environ Microbiol* 69(7):4167–4176.

21. Rontani JF, Bonin PC, Volkman JK (1999) Biodegradation of free phytol by bacterial communities isolated from marine sediments under aerobic and denitrifying conditions. *Appl Environ Microbiol* 65(12):5484–5492.

22. Oren A, Gurevich P, Anati D, Barkan E, Luz B (1995) A bloom of Dunaliella parva in the Dead Sea in 1992: biological and biogeochemical aspects. *Hydrobiologia* 297(297):173–185.

23. Perry GJ, Volkman J, Johns R (1979) Fatty acids of bacterial origin in contempary marine sediments. *Geochim Cosmochim Acta* 43:1715–1725.

24. Taylor J, Parkes RJ (1983) The Cellular Fatty Acids of the Sulphate-reducing Bacteria ,. *J Gen Microbiol* 129:3303–3309.

25. Ishige T, Tani A, Sakai Y, Kato N (2003) Wax ester production by bacteria. *Curr Opin Microbiol* 6(3):244–250.

26. Takano Y, et al. (2010) Sedimentary membrane lipids recycled by deep-sea benthic archaea. *Nat Geosci* 3(12):858–861.

27. Oren A (1999) Microbiological studies in the Dead Sea: Future challenges toward the understanding of life at the limit of salt concentrations. *Hydrobiologia* 405:1–9.

28. Finkelstein DB, Brassell SC, Pratt LM (2010) Microbial biosynthesis of wax esters during desiccation: Adaptation for colonization of the earliest terrestrial environments? *Geology* 38(3):247–250.
29. Neugebauer I, et al. (2016) Hydroclimatic variability in the Levant during the early last glacial (~ 117-75 ka) derived from micro-facies analyses of deep Dead Sea sediments. *Clim Past* 12(1):75–90.
30. Levy EJ, et al. (2017) Pore fluids in Dead Sea sediment core reveal linear response of lake chemistry to global climate changes. *Geology*:3–6.
31. Micić V, Köster J, Kruge MA, Engelen B, Hofmann T (2015) Bacterial wax esters in recent fluvial sediments. *Org Geochem* 89–90(October):44–55.
32. Rontani J-F, Bonin PC, Volkman JK (1999) Production of Wax Esters during Aerobic Growth of Marine Bacteria on Isoprenoid Compounds. *Appl Environ Microbiol* 65(1):221–230.
33. Van der Meer MT, et al. (2002) Alkane-1,2-diol-based glycosides and fatty glycosides and wax esters in Roseiflexus castenholzii and hot spring microbial mats. *Arch Microbiol* 178(3):229–237.
34. Van Der Meer MTJ, et al. (2010) Cultivation and genomic, nutritional, and lipid biomarker characterization of Roseiflexus strains closely related to predominant in situ populations inhabiting yellowstone hot spring microbial mats. *J Bacteriol* 192(12):3033–3042.
35. Cranwell P (1985) Esters of acyclic and polycyclic isoprenoid alcohols: biochemical markers in lacustrine sediments. *Adv Org geochemistry* 10:891–896.
36. Torfstein A, et al. (2015) Dead Sea drawdown and monsoonal impacts in the Levant during the last interglacial. *Earth Planet Sci Lett* 412:235–244.
37. Neugebauer I, et al. (2014) Lithology of the long sediment record recovered by the

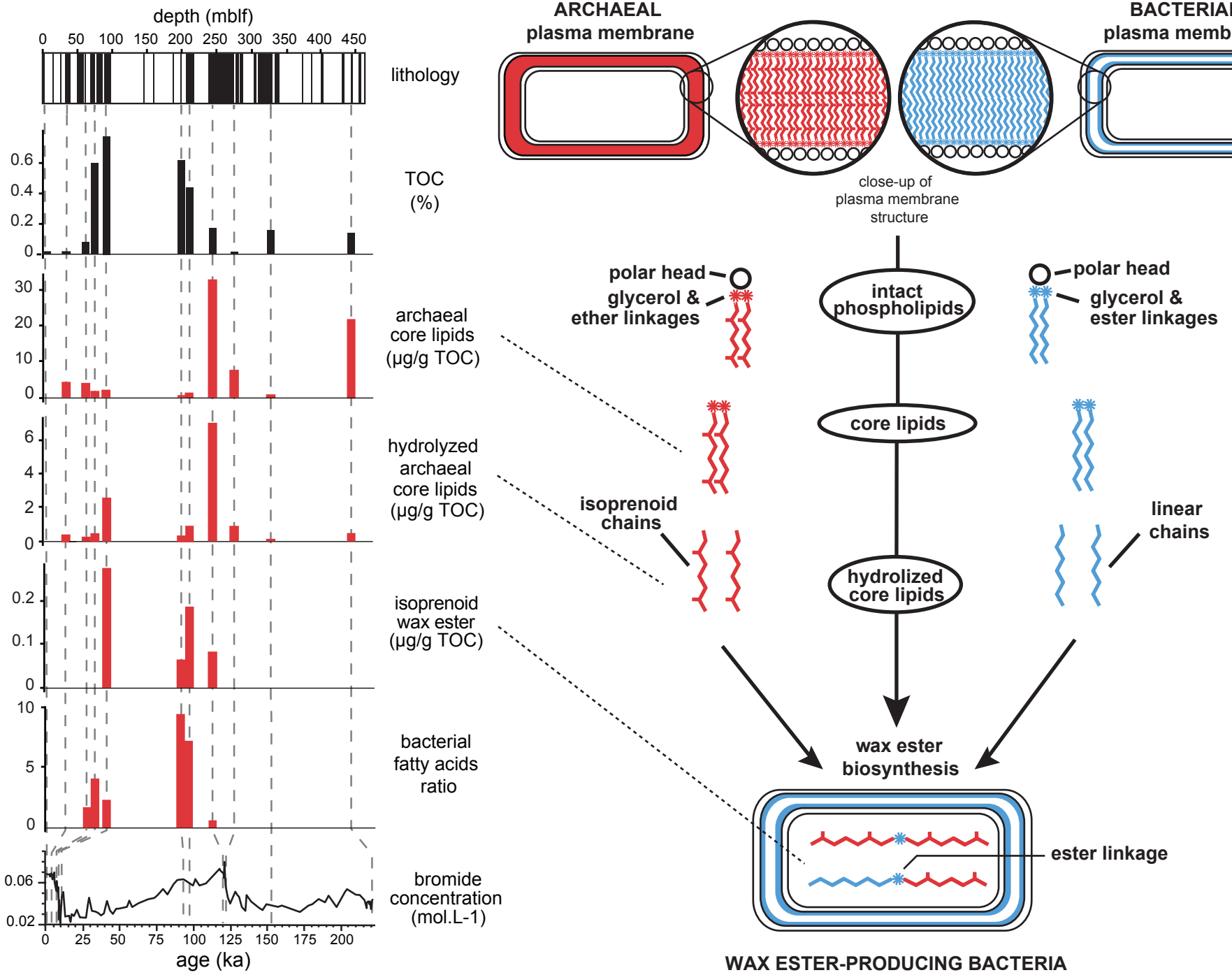
ICDP Dead Sea Deep Drilling Project (DSDDP). *Quat Sci Rev* 102:149–165.

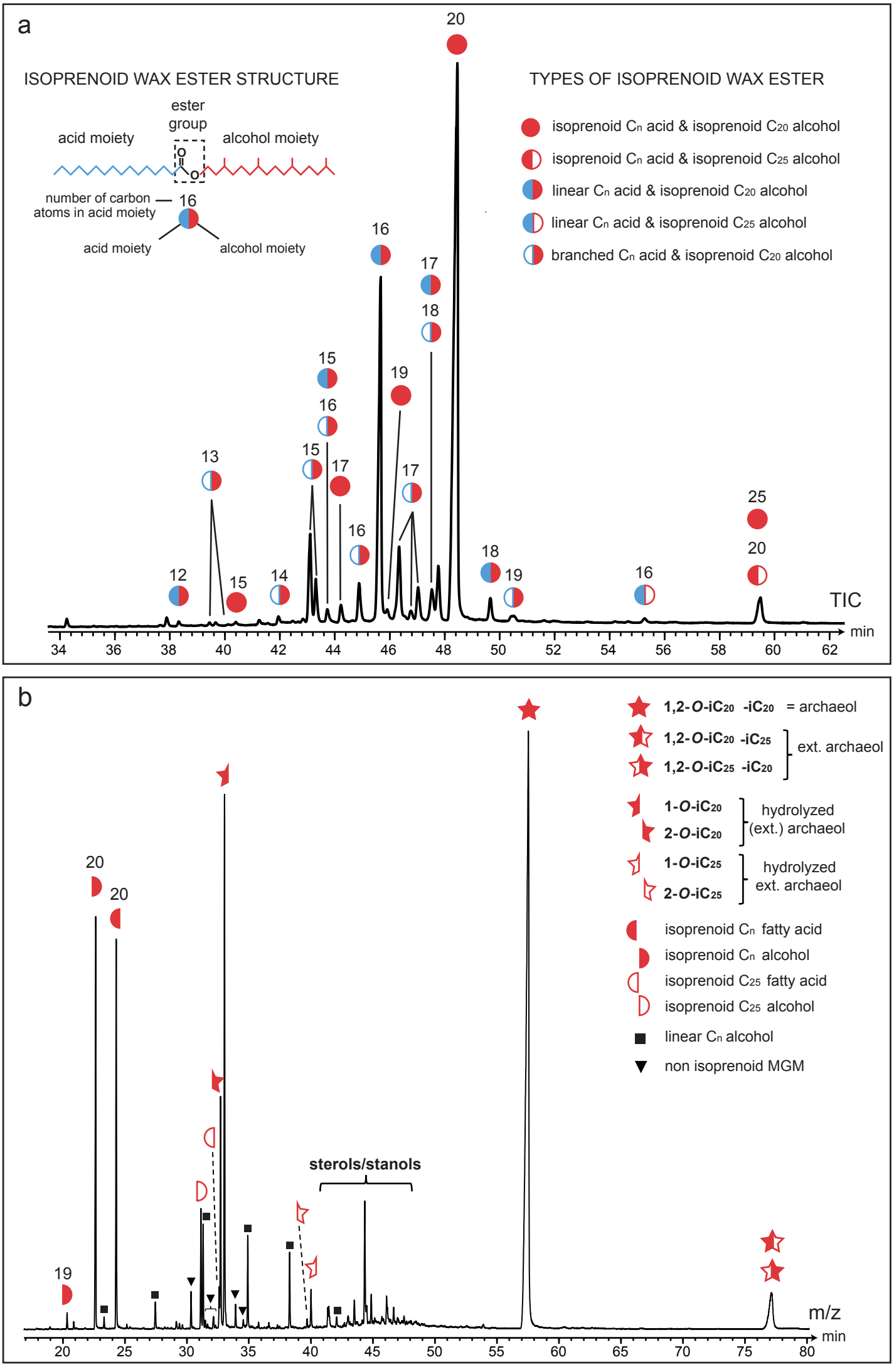
38. Kitagawa H, et al. (2016) Radiocarbon chronology of the DSDDP core at the deepest floor of the Dead Sea. *Radiocarbon* 59(November 2015):1–12.

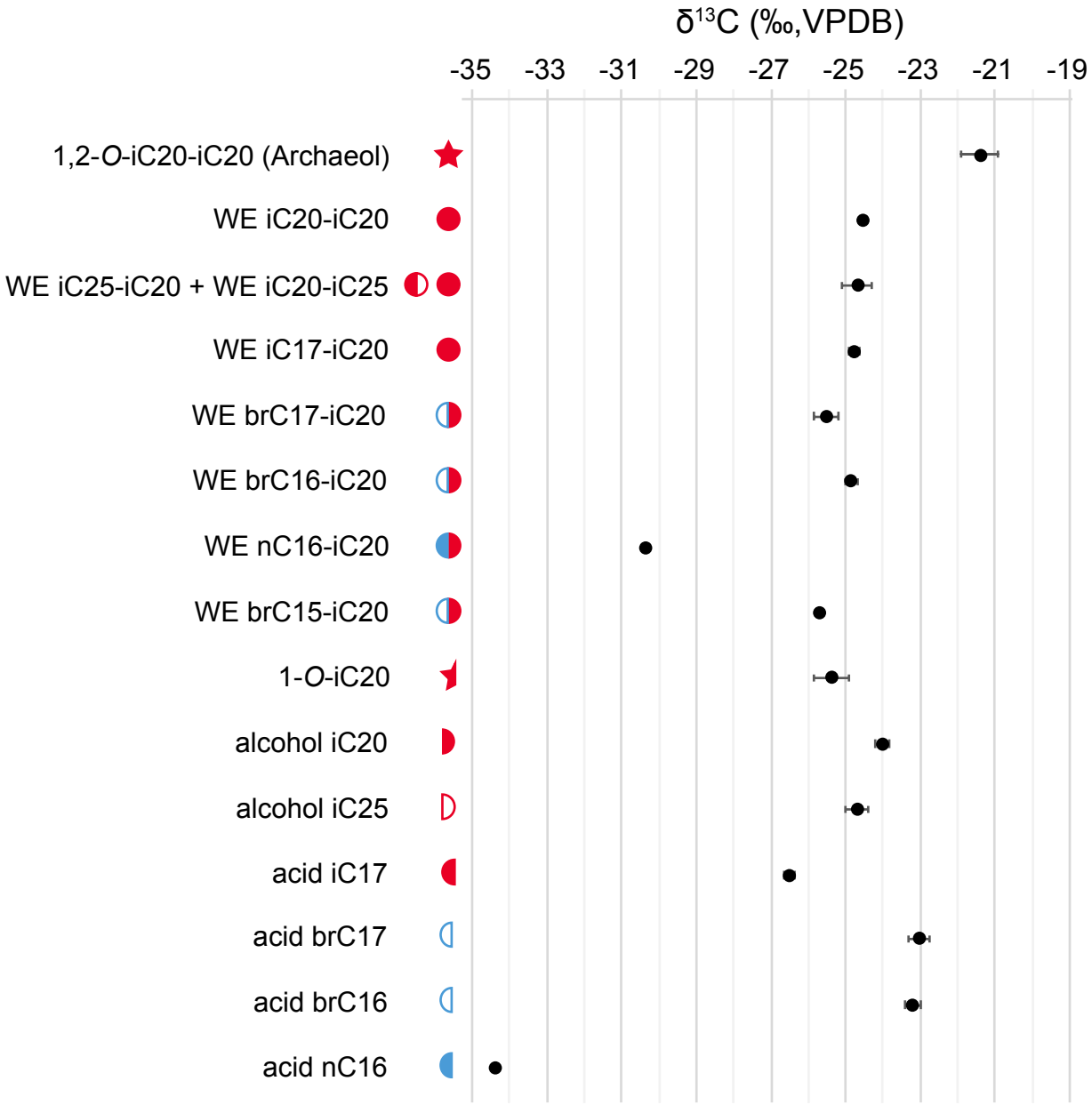
Fig. 1: Occurrence of wax esters (WE) and other lipid biomarkers along the Dead Sea core (gypsum and halite intervals are shown in black, dashed lines show the samples analyzed). The formation of the WE building blocks is schematized on the right-hand side of the panel and is linked to their quantitative profiles. The bacterial fatty acids ratio (sum of branched C_{15} and C_{17} over sum of linear C_{15} and C_{17} fatty acids) indicates the presence of bacteria likely involved in WE-production. The sampled layers are compared to bromide concentration in the sediment pore waters [used as a proxy for lake water dilution/concentration (30)] and fitted to ^{14}C ages (38) and U-ages (36). Red items indicate an archaeal origin, and blue items a bacterial origin.

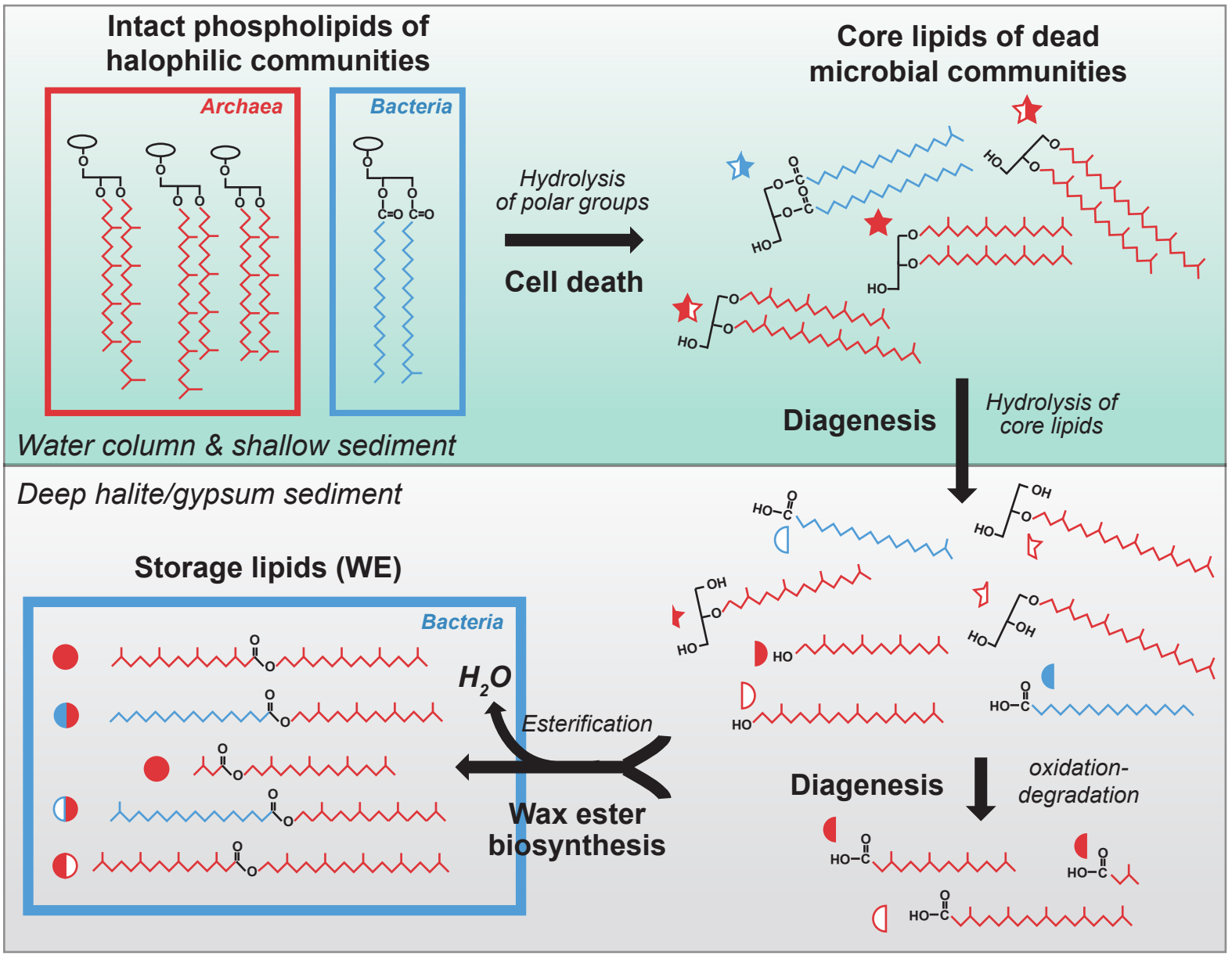
Fig. 2: Characteristic chromatograms of isoprenoid wax esters and related building blocks. (a) Total ion chromatogram (TIC) of the wax ester fraction from the gypsum sample at 91.04 mblf and (b) selected ions (m/z 103+133+159+205+218) chromatogram of a polar lipid fraction from the gypsum sample at 243.22 mblf. These intervals were selected because they show the highest concentrations of isoprenoid WE and hydrolyzed core lipids, respectively. MGM: monoalkyl glycerol monoethers. Red symbols indicate an archaeal origin and blue symbols a bacterial and/or eukaryal origin. The numbers above the symbols shown in (b) correspond to the number of carbon atoms in the chain.

406 Fig. 3: Compound specific stable carbon isotope composition of bacterial WE and their archaeal
407 and bacterial building blocks in a halite sample at 206.53 mblf. Symbols refer to the compounds
408 identified in Fig. 2.
409
410 Fig. 4: Schematic pathway for isoprenoid wax ester formation by bacterial recycling of
411 halophilic organism membrane lipids in Dead Sea sediments. Intact phospholipids from
412 archaeal and bacterial communities are degraded, and some of the degradation products are
413 transformed into WE by bacterial communities in the deep halite/gypsum sediment horizons.
414 Symbols refer to the compounds identified in Fig. 2. For the degradation products, moieties in
415 red have an archaeal origin and blue moieties have a bacterial origin.
416


depth (mblf)
0 50 100 150 200 250 300 350 400 450
lithology
TOC (%)
archaeal core lipids (µg/g TOC)
hydrolyzed archaeal core lipids (µg/g TOC)
isoprenoid wax ester (µg/g TOC)
bacterial fatty acids ratio
bromide concentration (mol.L-1)
0 25 50 75 100 125 150 175 200
age (ka)
ARCHAEAL plasma membrane
BACTERIAL plasma membrane
close-up of plasma membrane structure
polar head
glycerol & ether linkages
intact phospholipids
polar head
glycerol & ester linkages
core lipids
isoprenoid chains
linear chains
hydrolized core lipids
wax ester biosynthesis
ester linkage
WAX ESTER-PRODUCING BACTERIA


a
ISOPRENOID WAX ESTER STRUCTURE
ester group
acid moiety
alcohol moiety
number of carbon atoms in acid moiety
16
acid moiety
alcohol moiety
TYPES OF ISOPRENOID WAX ESTER
isoprenoid Cn acid & isoprenoid C20 alcohol
isoprenoid Cn acid & isoprenoid C25 alcohol
linear Cn acid & isoprenoid C20 alcohol
linear Cn acid & isoprenoid C25 alcohol
branched Cn acid & isoprenoid C20 alcohol
TIC
min
34 36 38 40 42 44 46 48 50 52 54 56 58 60 62
b
1,2-O-iC20 -iC20 = archaeol
1,2-O-iC20 -iC25
1,2-O-iC25 -iC20
ext. archaeol
1-O-iC20
2-O-iC20
hydrolyzed (ext.) archaeol
1-O-iC25
2-O-iC25
hydrolyzed ext. archaeol
isoprenoid Cn fatty acid
isoprenoid Cn alcohol
isoprenoid C25 fatty acid
isoprenoid C25 alcohol
linear Cn alcohol
non isoprenoid MGM
sterols/stanols
m/z
min
20 25 30 35 40 45 50 55 60 65 70 75 80


δ13C (‰,VPDB)
-35
-33
-31
-29
-27
-25
-23
-21
-19
1,2-O-iC20-iC20 (Archaeol)
WE iC20-iC20
WE iC25-iC20 + WE iC20-iC25
WE iC17-iC20
WE brC17-iC20
WE brC16-iC20
WE nC16-iC20
WE brC15-iC20
1-O-iC20
alcohol iC20
alcohol iC25
acid iC17
acid brC17
acid brC16
acid nC16


Intact phospholipids of halophilic communities
Archaea
Bacteria
Water column & shallow sediment
Hydrolysis of polar groups
Cell death
Core lipids of dead microbial communities
Diagenesis
Hydrolysis of core lipids
Deep halite/gypsum sediment
Storage lipids (WE)
Bacteria
H2O
Esterification
Wax ester biosynthesis
Diagenesis
oxidation-degradation