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**Fig. DR1: Compound specific stable carbon isotope composition of bacterial WEs and their archaeal and bacterial building blocks in a halite sample at 206.53 mblf. Symbols refer to the compounds identified in Fig. 3.**

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 WEs. 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 WEs 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 WEs. The $^{13}C$ composition of WEs 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. The slightly heavier $^{13}C$ composition of archaeol compared to archaeol hydrolysis products and isoprenoid WEs can be explained by the presence of different archaeal populations that may have developed asynchronously in the same sediment interval.

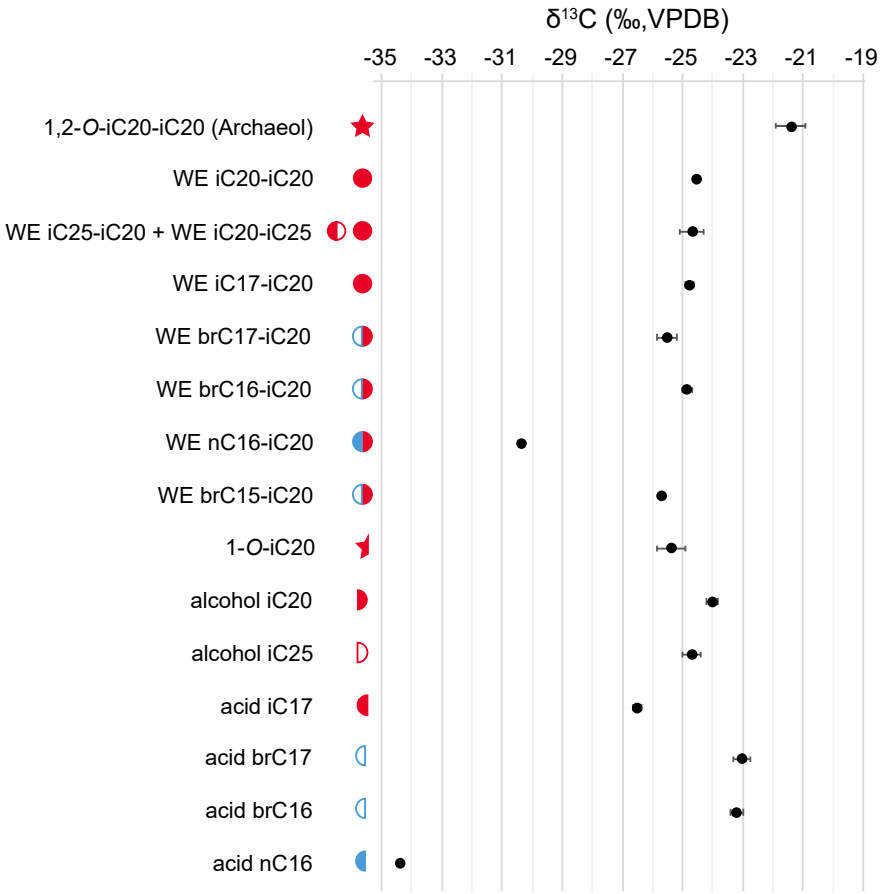
δ13C (‰,VPDB)
-35
-33
-31
-29
-27
-25
-23
-21
-19
1,2-O-iC20-iC20 (Archaeol)
WE iC20-iC20
WE iC25-iC20 + WE iC20-iC25
WE iC17-iC20
WE brC17-iC20
WE brC16-iC20
WE nC16-iC20
WE brC15-iC20
1-O-iC20
alcohol iC20
alcohol iC25
acid iC17
acid brC17
acid brC16
acid nC16

**Fig. DR2: Proposed pathway for the degradation of archaeal lipids and biosynthesis of bacterial isoprenoid wax esters in the Dead Sea sediments. *: not detected.**

The characterization of isoprenoid WEs has been shown to represent a useful tool for deciphering the metabolic pathways employed by microorganisms for the degradation of isoprenoid substrates (Rontani et al., 2003, 1999; Silva et al., 2007). The formation of isoprenoid WEs indeed allows the preservation of transient metabolites, which would not necessarily be preserved in their free form. Structural analysis of the different WEs present in the Dead Sea halite samples allowed us to decipher the metabolic pathways involved in the degradation of archaeal core lipids. Following hydrolysis of the ether linkages present in archaeol and extended-archaeol, a portion of the released isoprenoid $C_{20}$ and $C_{25}$ alcohol moieties was oxidized to the corresponding isoprenoid fatty acids, providing the building blocks for biosynthesis of $iC_{20}$-$iC_{20}$, $iC_{25}$-$iC_{20}$ and $iC_{20}$-$iC_{25}$ WEs. Part of the isoprenoid fatty acids was further degraded via successive α-oxidation, β-oxidation and β-decarboxymethylation sequences (Rontani et al., 1999; Silva et al., 2007), providing the acyl chains for shorter isoprenoid WE (Fig. 3a). The identification of $iC_5$-$iC_{20}$ WEs in some samples indicates the almost complete degradation of the isoprenoid alkyl chains released from the hydrolysis of archaeol and extended archaeol

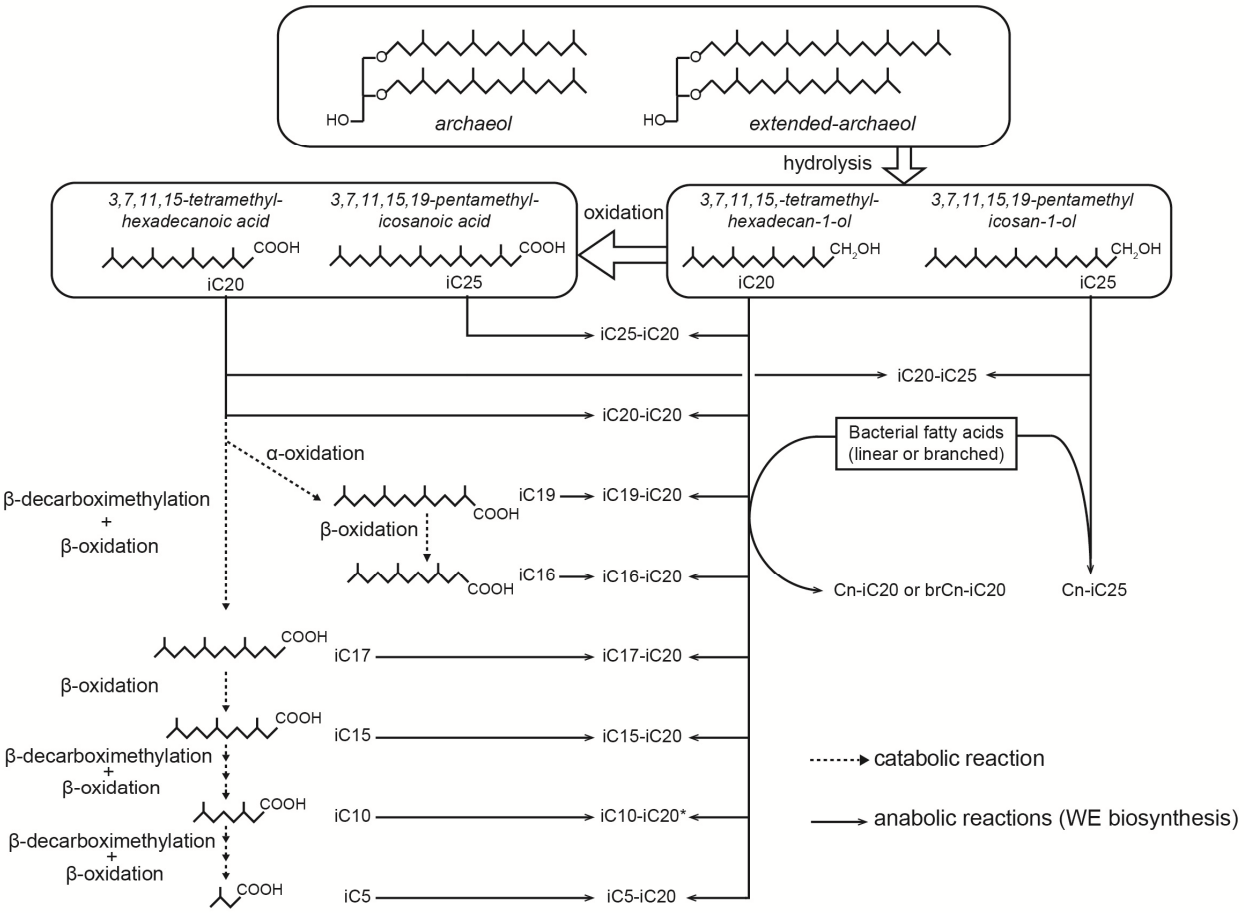
HO
archaeol
HO
extended-archaeol
hydrolysis
3,7,11,15-tetramethyl-hexadecanoic acid
COOH
iC20
3,7,11,15,19-pentamethyl-icosanoic acid
COOH
iC25
oxidation
3,7,11,15,-tetramethyl-hexadecan-1-ol
CH2OH
iC20
3,7,11,15,19-pentamethyl icosan-1-ol
CH2OH
iC25
iC25-iC20
iC20-iC25
iC20-iC20
Bacterial fatty acids (linear or branched)
α-oxidation
β-decarboximethylation + β-oxidation
iC19
iC19-iC20
β-oxidation
iC16
iC16-iC20
Cn-iC20 or brCn-iC20
Cn-iC25
iC17
iC17-iC20
β-oxidation
iC15
iC15-iC20
β-decarboximethylation + β-oxidation
catabolic reaction
iC10
iC10-iC20*
anabolic reactions (WE biosynthesis)
β-decarboximethylation + β-oxidation
iC5
iC5-iC20

**Table DR1: Sample characteristics and occurrence of isoprenoid wax esters in the Dead Sea sediments. Ages after $^{14}C$ dating by Neugebauer et al. (2014) and U-Th isochron (*) by Torfstein et al. (2015).**

| Sample | Depth (mblf) | estimated age | facies | Salinity (% TDS) | TOC (%) | isoprenoid wax esters |
|---|---|---|---|---|---|---|
| DS1 | 0 | present | halite | 38.8 | 0.01 | no |
| DS22 | 35.73 | 3.7-6.7 ka | halite | - | 0.01 | no |
| DS31 | 61.86 | 7.5-8.7 ka | gypsum (lamina) | 26.9 | 0.08 | no |
| DS19 | 76.76 | 8.7-11.4 ka | halite and detritus | 33.2 | 0.60 | no |
| DS21 | 91.04 | 11.4-14.5* ka | gypsum | 24.9 | 0.78 | yes |
| DS55 | 200.43 | 85*-95* ka | gypsum | 20.7 | 0.62 | yes |
| DS57 | 206.53 | 85*-95* ka | halite | 33.9 | 0.44 | yes |
| DS70 | 243.22 | 120* ka | gypsum | 32.9 | 0.17 | yes |
| DS107 | 273.71 | 120.5* ka | halite | - | 0.01 | no |
| DS96 | 327.43 | 155* ka | gypsum | 32.0 | 0.16 | no |
| DS176 | 444.65 | 242.6* ka | halite and detritus | - | 0.14 | no |

**Table DR2: Mass spectral characteristics of isoprenoid wax esters and archaeal lipid metabolites (as trimethyl silyl derivatives, TMS) detected in the Dead Sea sediments.**

| Compound | Fragments (relative intensity, %) | MW |
|---|---|---|
| **Wax ester** | | |
| *Isoprenoid acid - isoprenoid alcohol* | | |
| iC5-iC20 | 57(100), 71(58), 85(65), 103(47), 125(36), 280(4), 367(1), 382(1) | 382 |
| iC15-iC20 | 57(100), 71(88), 85(47), 125(41), 243(49), 280(17), 367(7), 522(4) | 522 |
| iC17-iC20 | 57(100), 71(80), 85(42), 125(43), 271(27), 280(18), 325(4), 550(4) | 550 |
| iC19-iC20 | 57(100), 71(81), 85(39), 125(37), 280(26), 299(34), 578(5) | 578 |
| iC20-iC20 | 57(100), 71(88), 85(50), 125(49), 280(27), 313(61), 325(4), 367(13), 592(5) | 592 |
| iC20-iC25 + iC25-iC20 | 57(100), 71(87), 85(48), 125(36), 280(13), 313(16), 350(5), 367(6), 383(25), 437(3), 662(3) | 662 |
| *Linear acid - isoprenoid alcohol* | | |
| C12-iC20 | 57(100), 71(68), 85(37), 125(42), 201(49), 280(16), 480(5) | 480 |
| C16-iC20 | 57(100), 71(80), 85(44), 125(54), 257(65), 280(22), 536(7) | 536 |
| C18-iC20 | 57(100), 71(79), 85(43), 125(48), 280(22), 285(55), 564(6) | 564 |
| C16-iC25 | 57(100), 71(86), 85(47), 125(47), 257(52), 350(13), 606(7) | 606 |
| **Isoprenoid alcohol (TMS)** | | |
| iC20 | 57(100), 71(64), 73(72), 75(72), 83(55), 97(55), 103(46), 111(46), 125(24), 355(36) | 370 |
| iC25 | 57(100), 71(62), 73(54), 75(53), 83(39), 97(38), 103(36), 111(33), 125(19), 425(20) | 440 |
| **Isoprenoid acid (TMS)** | | |
| iC20 | 55(35), 57(42), 73(100), 75(63), 117(98), 132(38), 143(49), 159(86), 369(39), 384(1) | 384 |
| iC25 | 55(33), 57(55), 73(89), 75(46), 117(93), 132(25), 143(32), 159(100), 439(28) | 454 |
| **Isoprenoid monoalkyl glycerol (TMS)** | | |
| 1-O-iC20 | 57(100), 71(47), 73(72), 85(25), 103(19), 147(40), 205(100), 369(5), 426(3), 501(2) | 516 |
| 2-O-iC20 | 57(100), 71(75), 73(80), 85(42), 103(45), 147(25), 191(15), 218(71), 412(4), 501(1) | 516 |
| 1-O-iC25 | 57(100), 71(43), 73(55), 85(24), 103(17), 147(34), 205(100), 439(2), 496(5), 571(1) | 586 |
| 2-O-iC25 | 57(100), 71(80), 73(78), 85(41), 103(16), 147(31), 191(15), 218(74), 425(14), 482(6) | 586 |

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