Molecular characterization of core lipids from halophilic archaea grown under different salinity conditions

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Halorhodobus utahensis, Natronomonas pharaonis, Haloferax sulfufoformis and Halobaculum gomorrense were grown at salinity values between 10% and 30% NaCl (w/v). The strains represent four haloarchaeal genera and have a range of salinity optima. Analysis of core membrane lipids of each strain using gas chromatography–mass spectrometry (GC–MS) revealed structures consistent with saturated, unsaturated and polyunsaturated dialkyl glycerol diethers (DGDs) including both phytanyl (C20) and sesterpanyl (C25) isoprenoid chains. In addition, we observed three trends related to salinity: (i) the proportion of unsaturated DGDs increased with increasing NaCl concentration in the medium, (ii) strains with a higher optimal NaCl concentration had a higher proportion of unsaturated DGDs and (iii) C25–20 DGDs occurred in the two strains with higher salinity optima, N. pharaonis and H. utahensis. The strong linear correlation between optimal growth salinity and fraction of unsaturated DGDs suggests that membrane lipid unsaturation is an important adaptation to specific salinity niches in archaeal halophiles. In addition, in three of the four strains, the fraction of unsaturated DGDs increased above a salinity threshold or in response to increasing salinity in the medium. Thus, haloarchaeal archaea regulate membrane lipid unsaturation in response to environmental salinity change, regardless of their salinity optima.

1. Introduction

Hypersaline water bodies, including salterns, soda lakes and the Dead Sea are characterized by a salt concentration ranging from 100 g l−1 to saturation. In addition to high salinity, these environments challenge resident biota because of their alkaline pH and high concentration of ions such as Mg2+. Despite the inhospitable conditions, hypersaline ecosystems are populated by diverse communities of halophilic algae, bacteria and archaea (Wilkinsky, 1936; Elazari-Volcani, 1943; Kamekura, 1998; Oren, 1999b; Ley et al., 2006).

Two physiological strategies exist for coping with the osmotic stress imposed by high salinity. Eukaryotes and most halophilic bacteria accumulate a high intracellular concentration of organic solutes (“organic-in” strategy) to balance the external osmotic pressure (Oren, 1999a). In contrast, halophilic archaea accumulate a high intracellular concentration of KCl, while excluding Na+ (“salt-in” strategy; van de Vossenberg et al., 1998; Oren, 1999a). Dialkyl glycerol diether (DGD) lipid membranes of halophilic archaea confer reduced membrane permeability to H+, Na+ and other solutes vs. bacterial fatty acid membranes (Choquet et al., 1992, 1994; Yamauchi et al., 1992; Elferink et al., 1994; van de Vossenberg et al., 1998; Tenchov et al., 2006). Thus a DGD membrane may be part of a successful “salt-in” strategy for balancing osmotic pressure in halophilic archaea. However, relatively little is known about the physiological advantage or ecological advantage that may be conferred by specific archaeal membrane lipid structures, including degree of unsaturation.

High salinity can be expected to have multiple effects on microbial membranes. Based on studies of bacterial and eukaryotic intact polar lipids (IPLs), increased salinity reduces the hydration of polar head groups, thereby decreasing membrane fluidity and potentially affecting other properties in ways that are poorly understood (Russell, 1989). A high concentration of salt or non-ionic polar solutes such as sucrose changes the geometry of bacterial IPLs in vitro, favoring a transition to non-lamellar (i.e. potentially membrane-disrupting) geometry for some IPLs. Significantly, the geometry adopted by IPLs in vitro is sensitive to interaction between head group and hydrocarbon tail properties such as their relative size, hydrocarbon unsaturation and substitutions, and head group charge (Russell, 1989). Most studies have focused on bacterial/eukaryal lipids, with the result that there are insufficient data to make clear predictions about salinity-related adaptation in archaeal membranes based on the chemical properties of archaeal isoprenoid IPLs. However, it is clear that both hydrocarbon and polar head group structures have a potential role to play in salinity adaptation via their effect on membrane properties.
The IPL composition of halophilic archaeal isolates shows some relationship, in particular with the type of the glycolipid distribution amongst genera (Kates, 1993; Asker and Ohta, 2002; Lattanzio et al., 2002; Oren, 2002; Oren et al., 2009) or the absence of glycolipids from haloalkaliphiles (Kates, 1993; Kamekura and Kates, 1999; Stadnitskaia et al., 2008), macrocyclic structures with up to two cyclopentane moieties (Comita et al., 1984; Stadnitskaia et al., 2003), OH substitutions (Stadnitskaia et al., 2008) and varying degrees of unsaturation in phytanyl chains (Gibson et al., 2005; Stiehl et al., 2005). Variation in core lipid composition thus has the potential to provide information about ancient hypersaline microbial communities and fluctuation in salinity in the past, although the utility of the approach remains to be explored.

Many studies of novel halophilic isolates report the major lipids on the basis of thin layer chromatography (TLC), with comparison to previously identified strains and authentic standards. This technique provides good resolution of the major IPLs [e.g. phosphatidylglycerol (PG), phosphatidylglycerophosphate (PGP), monogalactosyl glycerol diethers] and some information about core lipid chain length. Reports indicate that the halophilic archaeal genera Halobacterium, Haloarcula, Halofex, Halobaculum and Halorubrum contain (Table 1) only the C20–20 DGD structure (Oren et al., 1995; Kamekura and Kates, 1999; Asker and Ohta, 2002). The C25–20 DGD (extended archaeol; Table 1) occurs as the sole core lipid or, in addition to the C20–20 core lipid, in the genera Halococcus, Natronobacterium, Natronococcus, Natronorubrum, Natrinema, Natronorubrum, Haloterrigena and several other isolates with optimal pH > 7.0 (Tindall et al., 1984; Kamekura and Kates, 1999; Xu et al., 1999, 2001; Romano et al., 2007). However, in many other studies no data about core lipid structures are reported or, in the case of Halorhabdus utahensis, core lipids may have been misassigned as the C20–20 DGD (Waino et al., 2000). While IPL separation using TLC is most commonly used to characterize halophilic isolates, mild saponification followed by silylation and gas chromatography–mass spectrometry (GC–MS) provides more detailed and less ambiguous information about core lipid structures, as demonstrated by Stiehl et al. (2005). This approach can contribute to a better understanding of core lipid structures in archaeal physiology and enable a more robust interpretation of halophilic biomarkers, whether found in the modern environment or in the sedimentary record.

Membranes rich in unsaturated DGDs are common features of many archaeal halophiles. Minor amounts of monounsaturated archaeol were interpreted as an analytical artifact of hydroxyarchaeol extraction (Ekiel and Sprott, 1992). However, in other studies, isotopic differences between hydroxyarchaeol and monounsaturated archaeol (Blumenberg et al., 2005), as well as the presence of polysaturated archaeol in Halobacterium lacusprofundi and Methanococcoides burtoni (Franzmann et al., 1988; Nichols et al., 2004; Gibson et al., 2005) cannot be explained by OH loss. These studies therefore strongly suggest that unsaturated DGDs are not artifacts of sample preparation.

Although apparently common in archaeal halophilic membranes, the role of unsaturated core lipids in membrane adaptation to high salinity or other environmental conditions remains to be demonstrated. Adaptation to cold temperatures in Antarctic lakes may explain the polysaturated core lipid structures in H. lacusprofundi and M. burtoni (Nichols et al., 2004; Gibson et al., 2005). However, an alternative explanation is needed for the presence of highly unsaturated DGD lipids in many halophilic archaeal isolates from warmer environments (Upasani et al., 1994; Qiu et al., 1998; Gibson et al., 2005; Stiehl et al., 2005; de Souza et al., 2009). We hypothesized that unsaturated DGDs are a physiological response to osmotic stress in hypersaline environments. In order to test this hypothesis, we examined the impact of variation in salinity on the number of double bonds and proportion of unsaturated vs. saturated DGDs in the membranes of four species of halophilic archaea with a broad range of known salinity growth optima.

Table 1

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Optimal NaCl (%)</th>
<th>Optimal pH</th>
<th>C25–20</th>
<th>C20–20</th>
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<tr>
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<td>9</td>
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</table>
2. Material and methods

2.1. Microorganisms and culture conditions

_Haloferax sulfuriulentis_ strain SD1 (L. Krumholz, University of Oklahoma) was grown in the medium (pH 7.0) described by Elshahed et al. (2004), which contained (g l\(^{-1}\)): 150 g NaCl, 20 g MgCl\(_2\), 5 g K\(_2\)SO\(_4\), 0.1 g CaCl\(_2\) and 5 g yeast extract. _H. utahensis_ (DSMZ 12940) was grown in the medium (pH 7.6) described by Waino et al. (2000), which contained (g l\(^{-1}\)): 270 g NaCl, 0.1 g NaBr, 20 g MgSO\(_4\)\(_{7}\)H\(_2\)O, 5 g KCl, 2 g NaHCl, 12 g Tris–HCl, 0.125 g KH\(_2\)PO\(_4\). 0.05 g CaCl\(_2\)2H\(_2\)O, 5 mg FeCl\(_2\)4H\(_2\)O, 5 mg MnCl\(_2\)4H\(_2\)O, 0.5 g yeast extract and 2 g glucose. _Natronomonas pharaonis_ (DSMZ 3395) was grown at pH 9.0 in the medium described by Tindall et al. (1984), which contained (g l\(^{-1}\)) 200 g NaCl, 1 g KH\(_2\)PO\(_4\), 1 g NH\(_4\)Cl, 0.24 g MgSO\(_4\)\(_{7}\)H\(_2\)O, 0.17 g CaSO\(_4\)2H\(_2\)O, 5 g yeast extract, 1 g glucose, 5 g casamino acids, 5 g Na\(_2\)CO\(_3\), 1 ml trace element solution (g l\(^{-1}\)): 1.5 g FeCl\(_3\)-4H\(_2\)O, 100 mg MnCl\(_2\)-4H\(_2\)O, 70 mg ZnCl\(_2\), 6 mg H\(_2\)BO\(_3\), 190 mg CoCl\(_2\)-6H\(_2\)O, 2 mg CuCl\(_2\)-2H\(_2\)O, 24 mg NiCl\(_2\)-6H\(_2\)O, 36 mg Na\(_2\)MoO\(_4\)-2H\(_2\)O (pH 9.5). _Halobaculum gomorrense_ (DSMZ 9297) was grown at pH 7.0 in the medium described by Oren et al. (1995), which contained (g l\(^{-1}\)): 125 g NaCl, 160 g MgCl\(_2\)-6H\(_2\)O, 5 g K\(_2\)SO\(_4\), 0.1 g CaCl\(_2\)-2H\(_2\)O, 2 g soluble starch 1 g yeast extract, 1 g casamino acids. The media recipes gave the concentration of NaCl associated with optimal growth (salinity optima) as reported with strain descriptions (Tindall et al., 1984; Oren et al., 1995; Waino et al., 2000; Elshahed et al., 2004). All media were modified for the experiment to contain (l\(^{-1}\)) 100 g NaCl, 150 g, 200 g, 250 g and 300 g NaCl. Cells were harvested at late exponential phase by centrifugation.

2.2. Lipid extraction and analysis

Cell pellets were lyophilized prior to using a modified Bligh–Dyer extraction with MeOH/CHCl\(_3\)/H\(_2\)O (2:1:0.8) as described by Macalady et al. (2004). Hexacosane was added to samples as an internal standard to monitor recovery efficiency. An aliquot of the total lipid extract (TLE) was treated to remove neutral lipids including pigments, which would otherwise complicate GC–MS analysis of core lipids, by overnight precipitation in cold acetone (Kates et al., 1984). This method precipitates IPLs including pigments, which would otherwise complicate GC–MS analysis of core lipids from TLEs and acetone-precipitated IPLs from the four strains confirmed that the dominant membrane lipids were DGDs (Fig. 3), as expected from previous studies. Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown).

Separation was achieved with a Prevail Cyano column (2.1 × 150 mm 3 μm; Alltech, Deerfield, IL) at 30 °C. Glycerol diphtanyl glycerol tetaethers (GDGTs) were eluted isocratically with 99% hexane: 1% isopropanol for 5 min, followed by a linear gradient to 98.4% hexane: 1.6% isopropanol over 40 min. After each analysis the column was cleaned with 90% hexane: 10% isopropanol (10 min) followed by reequilibration (10 min) to 99% hexane: 1% isopropanol. The flow rate was 0.2 ml min\(^{-1}\). Instrument conditions included: corona 5000 nA, capillary 3500 V, nebulizer 60 psi, dry gas 61 min\(^{-1}\), dry temperature 200 °C, vaporizer temperature 400 °C. GDGTs were identified via comparison with lipids extracted from a laboratory culture of _Thermoplasma acidophilum_ containing GDGTs with zero to five cyclopentane rings.

3. Results

3.1. Identification of core lipids

Assignment of the C\(_{20}\)–C\(_{26}\) DGD (archaeol) and its unsaturated analogues (Fig. 1) followed MS fragmentation patterns published by Teixidor and Grimalt (1992), Teixidor et al. (1993) and Stiehl et al. (2005). Major MS fragments (m/z) of the archaeol trimethylsilyl (TMS) derivative include: M\(^+\) (724 m/z), M–C\(_{20}\)H\(_{41}\) (445 m/z), M–C\(_{20}\)H\(_{41}\)OH (426 m/z), M–C\(_{20}\)H\(_{41}\)OSi(CH\(_3\)_3) (369 m/z), C\(_{20}\)H\(_{41}\) (278 m/z) and CH\(_2\)(CH\(_2\))CH\(_2\OSi(CH\(_3\)_3) (130 m/z). The presence of unsaturation was observable in the shifting of the major ions to 2–8 m/z lower values and later elution times (Fig. 2). Assignment of C\(_{25–26}\) DGDs (extended archaeol), i.e. core lipids with a sterpenyl as well as a phytanyl chain (Fig. 1), was based on the following major MS fragments: M\(^+\) (794 m/z), M–C\(_{20}\)H\(_{41}\) (515 m/z), M–C\(_{20}\)H\(_{41}\)OH (497 m/z), M–C\(_{20}\)H\(_{41}\) (445 m/z), C\(_{20}\)H\(_{41}\) (348 m/z), C\(_{20}\)H\(_{41}\) (278 m/z) and CH\(_2\)(CH\(_2\))OSi(CH\(_3\)_3) (130 m/z). Again, the presence of unsaturation was observable in the downward shift of the major ions by 2–10 m/z values and later elution times (Fig. 2). The observed MS fragmentation is inconsistent with described macroyclic DGD structures (Comita et al., 1984; Stadnitskaia et al., 2003).

3.2. DGDs in halophilic archaeal isolates

GC–MS analysis of core lipids from TLEs and acetone-precipitated IPLs from the four strains confirmed that the dominant membrane lipids were DGDs (Fig. 3), as expected from previous studies. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown). Insufficient biomass was produced at 10% (w/v) NaCl for lipid analysis. No GDGTs were detected in any of the TLEs (data not shown).
pharaonis, at 15% (w/v) NaCl, 25% of DGDs contained unsaturation. The value increased to 45% at 30% (w/v) NaCl. In H. utahensis, during growth at 15% (w/v) NaCl, 62% of DGDs contained unsaturation. The value increased to 87% at 30% (w/v) NaCl. At all salinity values H. utahensis contained only C25–20 DGDs, with the unsaturated analogues consistently representing the dominant structure. N. pharaonis contained both C20–20 and C25–20 DGDs. H. sulfurifontis and H. gomorrense contained only C20–20 DGDs. Fig. 4 shows the fraction of unsaturated DGDs as a function of NaCl concentration for all strains. H. gomorrense showed an increase in unsaturated
DGDs above 15% (w/v) NaCl. There was no significant pattern in the average number of double bonds per DGD molecule with increasing salinity in the growth medium. The average fraction of unsaturated DGDs for each isolate across the full range of salinity tested shows a strong linear correlation with the strain-specific optimal salinity (Fig. 5). The linear regression describing the correlation is given by $y = 0.0049x - 0.6027$ ($y$ – average fraction unsaturated DGDs, $x$ – optimal NaCl, $R^2 = 0.9791$).

4. Discussion

We obtained GC–MS chromatograms and mass spectra for TMS-derivatized halophilic core lipids from acetone-precipitated IPLs of halophilic archaea grown at their strain-specific optimal salinity values. Cultures were grown between 10% and 30% NaCl (w/v). Halorhabdus utahensis (diamonds) makes solely $C_{20-25}$ DGDs and grows optimally at 27.5% NaCl (w/v). Natronomonas pharaonis (squares) makes both $C_{20-20}$ and $C_{20-25}$ DGDs and grows optimally at 20% NaCl (w/v). Haloferax sulfurifontis (triangles) and Halobaculum gomorrense (circles) makes solely $C_{20-20}$ DGDs and grows optimally at 15% NaCl (w/v) and 12.5% NaCl (w/v) respectively. Optimal NaCl concentration for each isolate is indicated by a vertical barbell. Error bars are ±1 standard deviation.

Fig. 3. Partial GC–MS chromatograms of TMS-derivatized core lipids from acetone-precipitated IPLs of halophilic archaea grown at their strain-specific optimal salinity values. Peaks eluting between 42 and 45 min are $C_{20-20}$ DGDs. Peaks eluting between 45 and 47.5 min are $C_{25-20}$ DGDs. Letter codes indicate structures from Fig. 1. Chromatograms for TLEs (no acetone precipitation step) were identical in terms of core lipid peaks, with relative peak areas not significantly different (±<10%) from the data shown.

DGDs above 15% (w/v) NaCl. There was no significant pattern in the average number of double bonds per DGD molecule with increasing salinity in the growth medium. The average fraction of unsaturated DGDs for each isolate across the full range of salinity tested shows a strong linear correlation with the strain-specific optimal salinity (Fig. 5). The linear regression describing the correlation is given by $y = 0.0049x - 0.6027$ ($y$ – average fraction unsaturated DGDs, $x$ – optimal % NaCl, $R^2 = 0.9791$).

4. Discussion

We obtained GC–MS chromatograms and mass spectra for TMS-derivatized halophilic core lipids after saponification of either TLEs (containing IPLs and neutral lipids such as pigments and squalene) or IPLs obtained following acetone precipitation to remove neutral lipids. These approaches should give approximately the same result with respect to core lipids, with simplified GC–MS chromatograms for samples that underwent acetone precipitation to remove neutral lipids. Because we did not determine double bond positions, we cannot say whether core lipids in the acetone-precipitated IPLs are identical to those in the TLE or not. However, core lipid peaks and their mass spectra in the two extracts were indistinguishable for all four isolates. Therefore we assume that core lipids derived from the acetone-precipitated IPLs are representative of the total IPLs with respect to number of unsaturations and isoprenoid chain length.

We found both saturated and unsaturated $C_{20-20}$ and $C_{25-20}$ isoprenoid DGDs in the four cultures, similar to compounds reported by Steihl et al. (2005) for Halobacterium marismortui, Haloferax

Fig. 4. Plot of total unsaturated archaeal $C_{20-20}$ and $C_{20-25}$ DGD lipids as a fraction of total DGDs in four halophilic archaea. Cultures were grown between 10% and 30% NaCl (w/v). Halorhabdus utahensis (diamonds) makes solely $C_{20-25}$ DGDs and grows optimally at 27.5% NaCl (w/v). Natronomonas pharaonis (squares) makes both $C_{20-20}$ and $C_{20-25}$ DGDs and grows optimally at 20% NaCl (w/v). Haloferax sulfurifontis (triangles) and Halobaculum gomorrense (circles) makes solely $C_{20-20}$ DGDs and grows optimally at 15% NaCl (w/v) and 12.5% NaCl (w/v) respectively. Optimal NaCl concentration for each isolate is indicated by a vertical barbell. Error bars are ±1 standard deviation.

Fig. 5. Average fraction of unsaturated DGDs vs. optimal % NaCl (w/v) for four halophilic archaeal strains. Error bars (±1 standard deviation) are contained within data points as drawn.
volcanii and Halorubrum sodomense, by de Souza et al. (2009) for Haloarcula marismortui and by Franzmann et al. (1988) and Gibson et al. (2005) for H. lacusprofundi. Analysis of the core DGD structures of the strains revealed three trends relating to salinity: (i) The unsaturated DGD proportion (unsaturated DGDs/total DGDs) in all the strains except H. gomorrense increased with increasing concentration of NaCl; (ii) the unsaturated DGD proportion was higher in strains with a higher optimal NaCl concentration, for example, in H. gomorrense [12.5% (w/v) NaCl optimum] 2.5–6% of DGDs were unsaturated vs. 62–87% in H. utahensis [27% (w/v) NaCl optimum]; (iii) C25–20 DGDs occurred in the strains with higher optimal NaCl concentration (N. pharaonis and H. utahensis). The C25–20 structure was the sole core DGD structure in H. utahensis, the strain with the highest optimal NaCl concentration.

The strong linear correlation between optimal growth salinity and fraction of unsaturated DGDs (Fig. 5) suggests that the degree of membrane lipid unsaturation is an important physiological adaptation to specific salinity niches. In addition, in three of the four halophile strains tested, the fraction of unsaturated DGDs increased above a salinity threshold or in response to increasing salinity in the medium. Based on these data, halophilic archaea appear to regulate membrane lipid unsaturation, increasing it in response to salinity increase, regardless of salinity growth optimum. Dannenmuller et al. (2000) showed evidence for increased membrane bilayer stability and decreased membrane permeability with the incorporation of a single double bond into each DGD phytanyl chain, consistent with unsaturation as a mechanism for decreasing membrane permeability in response to increased salinity. However, fully unsaturated phytanyl chains result in increased membrane permeability (Dannenmuller et al., 2000), indicating a more complex role of unsaturation on archaeal membrane permeability. Furthermore, permeability is only one of several membrane properties that may be affected by, and regulated in response to, salinity change. As noted above, salt appears to weaken the hydration of membrane polar head groups, decreasing membrane fluidity (Russell, 1989). Bacteria and fungi exposed to increased salinity have been shown to produce higher proportions of unsaturated fatty acids, leading to enhanced membrane fluidity (Russell, 1989; Turk et al., 2004). Unsaturated DGDs may represent a membrane adaptation in halophilic archaea as suggested by this study, as well as serving to enhance membrane fluidity in cold environments in strains such as H. lacusprofundi (Franzmann et al., 1988; Nichols et al., 2004; Gibson et al., 2005).

In contrast to DGD unsaturation, synthesis of C20–20 vs. C25–20 DGDs appears to be primarily related to taxonomy rather than pH or salinity optimum (Table 1). We note that, although C25–20 DGDs are generally associated with haloalkaliphiles (Kates, 1993; Xu et al., 1999), we identified C25–20 DGDs as the sole membrane lipids in Halorhabdus utahensis, a strain which grows optimally between pH 6.7 and 7.1. We also note an earlier report of this core lipid in the moderately acidophilic, halophilic archaeon Halarchaeum acidiphilum (Minegishi et al., 2010). Thus, the functional role of C25–20 DGDs in membrane adaptation to environmental conditions remains somewhat uncertain.

Studies of archaeal lipid membrane properties have shown that liposomes composed of GDGTs have reduced permeability vs. monolayer and bilayer GDGTs without the loss of membrane fluidity imposed by a GDGT monolayer (Russell, 1989). Extended archaeol with a mixture of C30 and C25 isoprenoid chains could thus provide reduced membrane permeability without significantly decreasing membrane fluidity. Since interaction between core lipids and head group chemistry could affect membrane properties in complex ways; this hypothesis should be tested in future work.

A novel hydroxylated C25–20 DGD structure was recently found in sediments from a saline cold seep (Stadnitskaia et al., 2008). The authors proposed that it represents a biomarker for archaeal methanotrophs at elevated salinity. The presence of extended hydroxyarchaeol as opposed to the C20–20 hydroxyarchaeol supports the role of C25–20 DGDs in providing decreased membrane permeability in hypersaline conditions. For halophile strains containing both core lipid structures, a response to salinity is suggested by an increase in the relative proportion of C25–20 to C20–20 with increasing salinity for Natronococcus occultus (Nicolaus et al., 1989), but such a pattern remains to be confirmed and was not observed here in the core lipids of N. pharaonis.

The utility of unsaturated DGDs as a paleosalinity proxy may be limited by the loss of double bonds during diagenesis. The relative proportion of extended archaeol offers a more readily preserved environmental indicator, similar to the salinity-driven ecologic signatures captured in the ratio of the saturated archaeol and caldarchaeol (Turich and Freeman, 2011). However, the degree of unsaturation could potentially offer greater insight into ancient biota and their habitat, as it records both community response and physiological response. In sulfidic environments, sulfurization of unsaturated hydrocarbons results in the reduction of double bonds and incorporation of sulfide (Sinninghe Damsté et al., 1989; Kohnen et al., 1991a,b), preserving information about the position and number of double bonds (Sinninghe Damsté et al., 1989). Sulfurization of unsaturated DGDs in ancient sediments can potentially preserve patterns such as those observed here, which would otherwise be lost due to the effect of diagenesis. Together with an enhanced understanding of the role of membrane core lipid structures in archaean halophile physiology, the analysis of such sulfurized biomarker compounds along with the relative proportions of archaeol, extended archaeol and caldarchaeol could provide new information about environmental conditions in the past.

5. Conclusions

Archaeal DGDs with 1–5 double bonds per core lipid were identified in four halophile strains. The unsaturated DGD analogues increased as a proportion of total DGDs with increasing NaCl concentration in the growth medium and with increasing salinity optimum across strains. Based on our results, unsaturation in halophilic archaeal membranes appears to be a physiological adaptation to salinity, both at the level of strain-specific salinity niches and physiological response to transient salinity changes. Unsaturation in DGD lipids may be a means of countering a salt-induced decrease in membrane fluidity while simultaneously reducing membrane permeability to ions as salinity increases. If so, archaeal membrane adaptation to salinity shows an important parallel with those suggested by studies of halotolerant and halophilic bacteria and eukaryotes. Unsaturated DGDs included analogues of both C20–20 and C25–20 core lipids. Although the presence or absence of C25–20 DGDs appears to be partly tied to taxonomy, the C25–20 structure may also provide decreased permeability that would be advantageous to archaea living in hypersaline environments.

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