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Tetraether-linked membrane monolayers in *Ferroplasma* spp: a key to survival in acid

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Abstract *Ferroplasma acidarmanus* thrives in hot, extremely low pH, metal-rich solutions associated with dissolving metal sulfide ore deposits. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and thin layer chromatography analyses of *F. acidarmanus* membranes indicate that tetraether lipids predominate, with at least three core lipid structures. NMR measurements indicate that the cytoplasmic pH of *F. acidarmanus* is ~5.6. The optimal growth pH is ~1.2, and the lowest growth pH is ~0.0. Thus, these organisms maintain pH gradients across their membranes that approach 5 pH units. Tetraether lipids were originally thought to be specifically associated with thermophiles but are now known to be widely distributed within the archaeal domain. Our data, in combination with recently published results for thermophilic and mesothermophilic acidophilic archaea, indicate that there may be a stronger association between tetraether lipids and tolerance to acid and/or large metal ion gradients.

Keywords Archaea · Acidophile · Acid mine drainage · Tetraether lipid · Membrane monolayer

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Introduction

Archaeal organisms of the Thermoplasmales family are important members of the microbial communities that populate hot, extremely acidic, metal-rich environments (Burton and Norris 2000; Vasquez et al. 1999). Two species of the genus *Ferroplasma* have been described to date: *F. acidiphilum* strains Y^T and Y-2 were isolated from acid bioleaching systems (Golyshina et al. 2000; Pivovarova et al. 2002), while *F. acidarmanus* strain fer1 was isolated from the Richmond ore deposit at Iron Mountain, Calif. (Edwards et al. 2000), and *F. acidarmanus* strains MT16 and MT17 from a bioleaching system (Okibe et al. 2003). *F. acidarmanus* can utilize organic carbon compounds, whereas *F. acidiphilum* appears to be a strict autotroph. Because both species oxidize ferrous to ferric iron (the primary oxidant of pyrite, FeS_2, under acid conditions), *Ferroplasma* spp probably play important roles in acid mine drainage generation, a serious and costly environmental problem worldwide.

F. acidarmanus is a champion extremophile. It is a common, and in some cases, the dominant organism in pyrite ore bodies and associated mine tailings and sub-aqueous slime streamer communities in 35–50°C, pH 0–1 solutions with approximately molar concentrations of metals and sulfate (Edwards et al. 1999).

The very high ionic strength of water surrounding the cells requires osmotic pressure regulation to prevent cytoplasmic water loss. Membrane adaptations which lower permeability to water and/or ions would contribute to osmotic regulation and prevent acidification of the cytoplasm. Because Thermoplasmales lineage organisms such as *Ferroplasma* spp lack cell walls, the cytoplasmic membrane represents the first line of defense against enormous ion concentration gradients, including proton gradients. As a result, the membrane properties of these organisms are likely to be key factors enabling their survival.

In this paper, we report the first characterization of the lipids of *F. acidarmanus*. The cytoplasmic pH in *F. acidarmanus* was determined as an initial step to understand the structural and physiological components of pH homeostasis in this obligate acidophile. *F. acidiphilum* lipids were also characterized to compare with the related *F. acidarmanus* strain and to confirm independently whether this organism has predominantly diethers (Golyshina et al. 2000) or predominantly tetraethers (Batrakov et al. 2002; Macalady et al. 2002).

Materials and methods

pH optimum for growth of *F. acidarmanus*

F. acidarmanus was grown aerobically at pH 1.0 at 37°C in medium described by Edwards et al. (1998) with some modifications. The medium contained (per liter): 20 g $FeSO_4{\cdot}7H_2O$, 800 mg $(NH_4)_2SO_4$, 800 mg $Ni(NH_4)_2$ $(SO_4)_2{\cdot}6H_2O$, 400 mg KH_2PO_4, 160 mg $MgSO_4{\cdot}7H_2O$, 85 mg $Na_2MoO_4{\cdot}2H_2O$, 70 mg $ZnCl_2$, 31 mg H_3BO_3, 10 mg $MnCl_2{\cdot}4H_2O$, 10 mg $CoCl_2{\cdot}6H_2O$, and 0.1% (w/v) yeast extract (Becton Dickinson, Sparks, Md.). Chemicals were obtained from Sigma/Aldrich Chemical Co. (St. Louis, Mo.) except for $Ni(NH_4)_2$ $(SO_4)_2{\cdot}6H_2O$, KH_2PO_4, and $(NH_4)_2SO_4$ (Mallinckrodt, Hazelwood, Mo.) and H_3BO_3 (EM Science, Gibbstown, N.J.). The medium was prepared by adding all ingredients except $FeSO_4$ and yeast extract to 1 l of H_2O and adjusting to a pH of 1.0, using concentrated H_2SO_4. All pH measurements were made using a Ross Orion glass electrode and an Accumet AR10 pH meter (Fisher Scientific, Pittsburgh, Pa.), calibrated using fresh standards (pH 7.0, pH 1.0; Fisher Scientific, Pittsburgh, Pa.) and 0.146 M (pH 0.86), 0.734 M (pH 0.09), and 1.497 M (pH −0.38) H_2SO_4 reference solutions (Edwards et al. 2000). The basal salts solution was sterilized for 25 min at 121°C and 103 kPa. Yeast extract was added to deionized water and sterilized separately. Prior to inoculation, $FeSO_4$ and yeast extract were added to 100 ml of the autoclaved medium and then filter-sterilized, using a 0.2-μm pore membrane filter (Fisher Scientific, Pittsburgh, Pa.) to eliminate precipitation caused by acidification of yeast extract. Following the addition of the 100 ml of $FeSO_4$ and yeast extract solution to 900 ml of the basal medium (1 l final), the medium was inoculated with 20 ml of 7-day culture. The inoculated medium (200 μl) was distributed into the wells of a sterile multi-well plate and growth was monitored spectrophotometrically (optical density at 492 nm; OD_{492}), using a Bioscreen C analyzer (Thermolabsystems, Helsinki, Finland). Background OD_{492} values for uninoculated medium (negligible in most experiments) were subtracted from the OD_{492} values of cultures.

Viable cell counts

Attempts to grow *F. acidarmanus* on a solidified medium were unsuccessful, therefore viable cell counts were determined by a 3-tube most probable number (MPN) technique (Gerhardt et al. 1981). The inoculated tubes were incubated at 37°C for 60 days (generation time is ca. 2 days; therefore, one cell would multiply to 10^9 cells/ml after approximately 60 days). Tubes were checked visually for growth and the MPN/ml calculated.

Intracellular pH measurements

Several methods are available for measuring intracellular pH in living cells, but nuclear magnetic resonance (NMR) spectroscopy remains the only non-invasive technique (Lundberg et al. 1990). Moon and Richards (1972) utilized NMR to monitor inorganic phosphate ^{31}P in cells to ascertain cytoplasmic or internal pH. The biggest challenge with the application of NMR to measure the intracellular pH of prokaryotes is their reduced internal volume in comparison with eukaryotic cells. To circumvent this problem with *F. acidarmanus*, methods were established to grow cultures to ca. 10^{10} cells/ml and to obtain approximately 8×10^{13} viable cells for NMR measurements.

NMR measurements on cells of *F. acidarmanus* were recorded on a Bruker DMX-500 (11.52 T) spectrometer operating at 161.972 MHz. ^{31}P NMR spectra were acquired in a 5-mm NMR glass tube (Wilmad/Lab glass, Buena, N.J.) with a 30° pulse (800 ms), with an acquisition time of 12 h. Cells from 8 l of media were harvested after 7 days of incubation by centrifugation at 13,180 *g* for 15 min. After collection, cells were resuspended and washed twice with 1% NaCl adjusted to pH 1.0, using concentrated sulfuric acid. The washed cells were resuspended in 0.6 ml of acidified 1% NaCl and analyzed. Chemical shifts were reported in parts per million (ppm), using 1 M phosphoric acid as a reference. The equation used for calculating the intracellular pH was: $pH = 6.77 + \log[(\text{Pi shift} - 3.29)/(5.68 - \text{Pi shift})]$, where Pi shift is the chemical-shift difference between phosphocreatine (PCr) and inorganic phosphate (Pi) resonances. The resonance value used for PCr was −2.55.

Statistical analysis

Data were analyzed for statistical differences using the *t*-test and SigmaPlot software (Jandel Scientific, San Rafael, Calif.).

Preparation of membrane lipids

For mass spectroscopy (MS), *F. acidarmanus* strain fer1 cultures were grown at pH 1.2 in modified 9 K media, as reported by Edwards et al. (2000). For thin layer chromatography (TLC), *F. acidarmanus* was grown as described above, except with a higher yeast extract concentration (2 g/l). As growth yields were higher in the presence of high yeast extract concentration, it is likely that *F. acidarmanus* was growing heterotrophically in this medium. *F. acidophilum* strain Y^T (DSMZ 12658) was grown at pH 1.7 and 37°C in DSMZ 874 medium without shaking. The lipids of *T. acidophilum* (DSMZ 1728) were prepared for use as tetraether standards. *T. acidophilum* was grown at pH 1.65 and 59°C in DSMZ 158 medium with shaking at 90 rpm. A *Halorubrum saccharovorum* total lipid extract was provided by Dr. Linda Jahnke, NASA Ames Research Center, Ames, Calif.

Logarithmically growing cells were harvested from 10 l of growth medium, washed, and resuspended in acidified 1% NaCl. Lipids were extracted from cell pellets using methods described by Hedrick and White (1995). Briefly, total lipid extracts were prepared by incubating overnight with $CHCl_3/CH_3OH/H_2O$ (1.0:2.0:0.8 by vol.). Total lipid extracts were fractionated according to polarity by column chromatography on silica gel columns (0.50 g Si; Supelco, Bellefonte, Pa.). Core lipids were prepared from polarity fractions and lipid-extracted cell residues by strong acid hydrolysis (2 h at 100°C in $CHCl_3/CH_3OH/HCl$; 10:1:1 by vol.).

Lipid analysis

Total lipid extracts and core lipids were analyzed by delayed extraction reflectron matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS; Bruker Reflex II, 337-nm nitrogen laser) using 2′,4′,6′-trihydroxyacetophenone as a matrix. The instrument was calibrated using 1,2-diether-hexadecyl-rac-glycero-3-phosphocholine (Sigma) and a mixture of three peptides (Sigma). Diether and tetraether standards were analyzed under the same conditions as unknowns.

Core lipids from *F. acidarmanus*, *T. acidiphilum* (tetraether standard; Shimada et al. 2002; Swain et al. 1997), and *H. saccharovorum* DSMZ 1137 (diether standard; Lanzotti et al. 1988) were also characterized by TLC on Whatman LKDG silica gel plates (60 A, 250 μm layer thickness). Core lipids were developed in hexane:ethylacetate (7:3 v/v; Trincone et al. 1988), allowing separation of diethers and tetraethers with 0–4 rings per isoprenoid chain, and detected by misting plates with 40% (v/v) aqueous H_2SO_4 and baking them at 100°C for 1 h.

Results

Optimal growth pH

F. acidarmanus grew in the pH range 0.0–1.5, with a maximum OD_{492} at day 7 (Fig. 1). Interpretation of the optimal growth pH was complicated by atypical growth curves exhibiting two phases, resembling diauxic growth. Optimal growth was observed between pH 0.6 and pH 1.4. Growth at $pH < 0.4$ and $pH > 1.4$ was significantly less ($P < 0.05$) when compared with cultures grown in the optimal range. The maximum OD_{492} at day 7 in the optimal pH range did not differ significantly ($OD_{492} = 0.220 \pm 0.004$). At pH 0.0, growth was not evident from spectrophotometric measurements until day 21 of incubation. These data indicate that the pH gradient across *F. acidarmanus* membranes may approach > 5 pH units.

Intracellular pH

The intracellular pH was obtained by NMR as the chemical shift between the PCr and Pi resonances. The pH was calculated using the Henderson–Hasselbach equation and the calibration curve determined by Petroff et al. (1985). The average value obtained for fresh cells of *F. acidarmanus* (tested within 3 h of harvesting) was pH 4.9 ± 0.48 ($n = 3$). The use of fresh cells was necessary

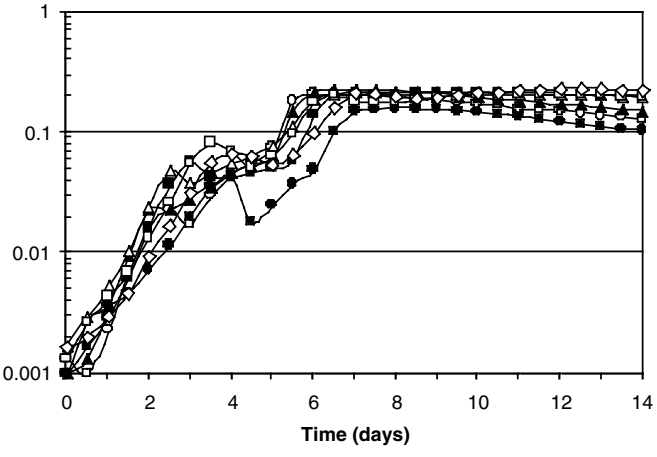

Fig. 1 Growth of *F. acidarmanus* at pH 0.2 (*closed circles*), pH 0.4 (*open circles*), pH 0.6 (*closed triangles*), pH 0.8 (*open triangles*), pH 1.0 (*closed squares*), pH 1.2 (*open squares*), and pH 1.4 (*open diamonds*) at 37°C. The background absorbance of uninoculated medium was subtracted from OD_{492} values. Each point represents the mean from three independent trials

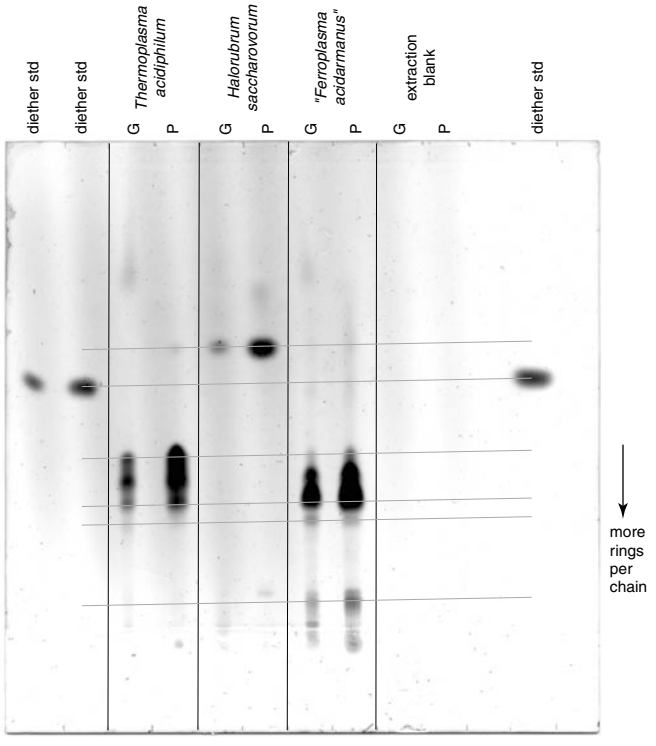

Fig. 2 TLC for core lipids of *F. acidarmanus* and diether and tetraether standards. Core lipids derived from glycolipid (*G*) and polar lipid (*P*) fractions of all cultures are shown. Core lipids from glycolipid and polar lipid fractions of *F. acidarmanus* consistently showed a low-mobility band not present in *T. acidiphilum*. *std* Standard

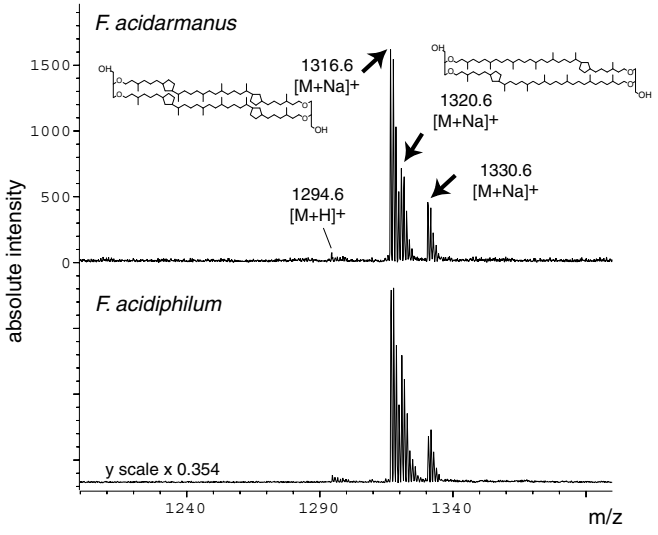

Fig. 3 Positive-ion MALDI-TOF spectra for acid-hydrolysed lipid extracts of *F. acidarmanus* (*top*) and *F. acidiphilum* (*bottom*, *y*-axis scaled by 0.354). Core lipids in the two strains are effectively identical. Core lipids were detected primarily as their sodium ion adducts ($[M+Na]^+$). The largest peaks in both spectra (m/z 1,293.6, m/z 1,297.6) are consistent with the previously identified tetraether core structures shown. Cyclopentane rings are drawn based on analogy with structures in the literature (see Batrakov et al. 2002; Shimada et al. 2002; references therein). The structure of the third major core lipid (m/z 1,307.6) was not determined. No peaks consistent with diether cores were detected

because cells that were stored at 4°C (18–24 h) prior to analysis had an intracellular pH of 6.03 ± 0.48 ($n = 5$, data not shown).

Lipids

Total lipid extracts accounted for 9% of the dry weight of *F. acidarmanus* cells. Figure 2 shows TLC results for core lipids in glycolipid (acetone-soluble) and polar lipid (methanol-soluble) polarity fractions of *T. acidiphilum*, *H. saccharovorum*, and *F. acidarmanus* lipids. The *F. acidarmanus* lipids detected by TLC are tetraethers. No diethers were observed. On average, the *F. acidarmanus* lipids contain more rings per chain than *T. acidiphilum*. *F. acidarmanus* lipids also contain a low-mobility core lipid not present in *T. acidiphilum*. No core lipids were detected in acid-hydrolysed cell residues from total lipid extracts of *F. acidarmanus*. Small amounts of lipid were detected in acid-hydrolysed neutral lipid fractions from both *F. acidarmanus* and *T. acidiphilum* (data not shown). These compounds were not likely to be derived from membrane lipids and were not analyzed further.

Figure 3 shows MALDI-TOF spectra for core lipids from both *Ferroplasma* species. No diether cores were detected. Three distinctive tetraether core lipids are resolved in the spectra, and these are identical for both *Ferroplasma* species. Two of the tetraether core lipids have m/z consistent with previously identified tetraether core lipids having one and two cyclopentane rings per chain (Hopmans et al. 2000). Core lipid structures shown in Fig. 3 are assigned based on comparison with *T. acidiphilum* core lipids with known structures, using the same analytical conditions (data not shown; Swain et al. 1997). The mass of the third core lipid ($[M+Na]^+$ $m/z = 1{,}330.6$) in both *Ferroplasma* species spectra is not consistent with previously identified core lipid structures.

Figures 4, 5 show MALDI-TOF spectra for total lipid extracts from *F. acidarmanus* and *F. acidiphilum*, respectively. The most abundant compounds in the spectra have molecular masses between 1,400 and 1,800, within the range expected for known tetraether lipids. Minor peaks corresponding to molecules with masses of 700–1,000 indicate the possible presence of diether lipids. Although the core lipid types from the two species are identical, the spectra obtained from unhydrolysed total lipid extracts differ significantly (Figs. 4, 5), indicating that different head groups are present.

Discussion

Based on TLC and MALDI-TOF-MS analyses, *F. acidarmanus* membranes are composed largely or entirely of

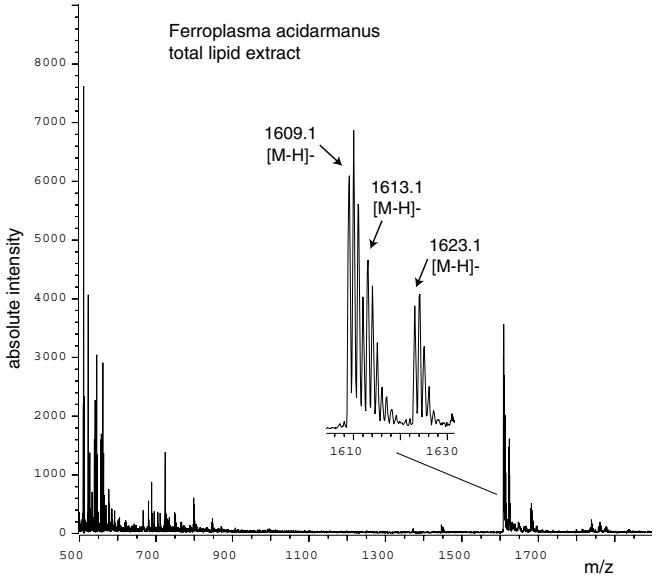

Fig. 4 Negative-ion MALDI-TOF spectrum for total lipid extract of *F. acidarmanus* strain fer1. Known archaeal tetraether membrane lipids have m/z between 1,400 and 2,000. *Inset* shows isotopic resolution of peaks for the most abundant compounds detected. Based on the MALDI run conditions and the expected isotope distributions for known tetraether lipid compounds, the peaks shown in inset represent at least three compounds with molecular masses differing by 4 m/z and 14 m/z units (indicated by *arrows*). This pattern is also evident in spectra for core lipids (head groups removed) shown in Fig. 3. Smaller peaks between m/z 700 and m/z 1,000 are consistent with known diether lipid structures

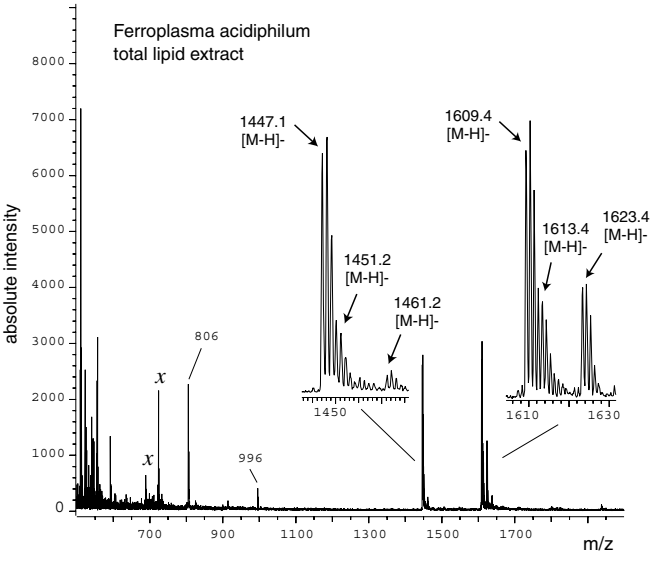

Fig. 5 Negative-ion MALDI-TOF spectrum for total lipid extract of *F. acidiphilum*. Known archaeal tetraether compounds have m/z between 1,400 and 2,000. Insets show isotopic resolution of peaks for the most abundant compounds, which display a pattern of mass distribution similar to that identified in *F. acidarmanus* within major peaks (Fig. 4). Smaller peaks between m/z 700 and m/z 1,000 are consistent with diether lipid structures. Peaks annotated with the letter *x* were detected in blanks

tetraethers, including at least three major tetraether core lipid structures. Analyses of the related extreme acidophile *F. acidiphilum* confirmed that its membranes are also dominated by tetraethers (Batrakov et al. 2002; Macalady et al. 2002), rather than diethers (Golyshina et al. 2000). MALDI-TOF-MS spectra for core lipids of both *Ferroplasma* species were identical (Fig. 3), suggesting that the two organisms are closely related despite differences in carbon catabolism and pH optima (pH 1.1 for *F. acidarmanus*; pH 1.7 for *F. acidiphilum*; Golyshina et al. 2000). Two of the core lipids have exact masses corresponding with caldarchaeol modified by the addition of one or two cyclopentane rings per chain, respectively ($[M+Na]^+$ $m/z=1{,}320.6$, $m/z=1{,}316.6$). Caldarchaeol containing one cyclopentane ring per chain is reported to be the core lipid of the most abundant membrane lipid in *F. acidiphilum* (Batrakov et al. 2002). The mass spectrum of the third tetraether core ($[M+Na]^+$ $m/z=1{,}330.6$) is not consistent with previously reported tetraether structures and is currently being characterized further.

Some archaeal lipids contain hydroxy-substituted cores which would be modified or destroyed by the strong acid hydrolysis step used to prepare core lipids from membrane lipids (Sprott et al. 1990). Hydroxy-substituted cores have not been reported in thermoacidophilic archaea. More importantly, the distinctive MALDI-TOF-MS isotopic pattern evident in spectra for lipid cores prepared by strong acid hydrolysis (Fig. 3) is also present in spectra for intact membrane lipids not subjected to this procedure (Figs. 4, 5), strongly suggesting that no hydroxy substitutions are present in *Ferroplasma* spp membrane core lipids.

The peak at $m/z=1{,}613.4$ in the mass spectrum for *F. acidiphilum* total lipid extract (Fig. 5) is consistent with the structure of β-D-glucopyranosyl caldarchaetidylglycerol, reported as the main membrane lipid in this organism (Batrakov et al. 2002). This is also a major peak in the mass spectrum for *F. acidarmanus* lipids (compare Figs. 4, 5). Based on the mass spectra obtained for core lipids of both species and the isotopic pattern evident in spectra for both cores and total lipid extracts, both organisms likely synthesize significant amounts of each of the three core lipids with head group configurations analogous to β-D-glucopyranosyl caldarchaetidylglycerol. Confirmation of this prediction awaits further characterization of *F. acidarmanus* membrane lipids.

The number of rings per isoprenoid chain in lipids of thermoacidophilic archaea is thought to be an important mechanism by which these organisms "tune" their membrane properties to temperature (Gabriel and Chong 2000; Uda et al. 2001). Based on TLC analyses (Fig. 2), *T. acidiphilum* lipids (pH 1.65, 59°C) contain fewer rings per chain than *Ferroplasma* spp lipids (pH 1.2–1.7, 37°C). This result indicates that other membrane structures and/or physiological adaptations are also important aspects of membrane adaptation to environmental conditions. Mass spectra

Table 1 Temperature optima, pH optima, and percentage of tetraethers in core lipids for archaeal isolates. Percentage values are ±10% and reflect minimum tetraether content in studies where lipids were not completely quantified. Table entries ($n = 49$ isolates) were used to construct Fig. 6

Genus and species	pH optimum	Temperature optimum (°C)	Tetraethers (%)	References
Ferroplasma "acidarmanus"	1.1	37	100	This study
F. acidiphilum	1.7	35	100	This study; Batrakov et al. (2002)
Picrophilus oshimae	0.7	60	100	Schleper et al. (1995)
P. torridus	0.7	60	100	Schleper et al. (1995); van De Vossenberg et al. (1998)
Thermoplasma acidiphilum	1.0–2.0	59	95	Shimada et al. (2002)
T. volcanium	2	55	95	Swain et al. (1997)
Archaeoglobus fulgidus	5.5–8	60–95	Minor	Kessel et al. (1990)
Palaeococcus ferrophilus	6	83	>80	Takai et al. (2000)
Pyrococcus woesei	6	101.5	100	Lanzotti et al. (1989)
Thermococcus celer	5.8	88	0	De Rosa et al. (1987)
T. hydrothermalis	7	85	55	Lattuati et al. (1998)
Methanobrevibacter smithii	6.9–7.4	35	34	Sprott et al. (1999)
Methanocaldococcus jannaschii	6	85	0	Comita et al. (1984); Ferrante et al. (1990)
Methanococcus voltae	6.5–8	<20–45	0	Ferrante et al. (1986)
Methanococcoides burtonii	7–7.8	20–23	0	Nichols and Franzmann (1992)
Methanosaeta concilii	7.3	35	0	Ferrante et al. (1988)
Methanosphaera stadtmanae	6.5–6.9	30–40	8	Sprott et al. (1999)
Methanothermus fervidus	6.5	80–85	>91	Morii et al. (1998)
Methanosarcina barkeri	6.7	35	Trace, 0	De Rosa et al. (1986); Sprott et al. (1994a)
M. mazei	6.7	35	0	Sprott et al. (1994a)
M. thermophila	6.7	50	0	Sprott et al. (1994a)
Methanopyrus kandleri	6.5	97	Minor, 0	Nishihara et al. (2002); Sprott et al. (1997)
Methanospirillum hungatei	6.6–7.4	30–45	>46	Sprott et al. (1994b)
Haloarcula quadrata	7	35–40	0	Oren et al. (1999)
Halobacterium salinarum	7	35–50	0	Corcelli et al. (2000); Qiu et al. (2000)
Halobaculum gomorrense	7 to 8	40	0	Oren et al. (1995)
Halobiforma haloterrestris	7	42	0	Hezayen et al. (2002)
Haloferax volcanii	7	40	0	Lanzotti et al. (1988)
H. mediterranei	6.5	53	0	Lanzotti et al. (1988)
Halorubrum saccharovorum	7	50	0	Lanzotti et al. (1988)
Haloterrigena thermotolerans	7	50	0	Montalvo-Rodriguez et al. (2000)
Halorubrum tebenquichense	7	40	0	Lizama et al. (2002)
Natrialba asiatica	6.6–7.8	35–40	0	Hezayen et al. (2002)
N. magadii	9.5	37–40	0	Qiu et al. (1998)
Natronomonas pharaonis	9.5–10	45	0	Tachibana 1994)
Natronorubrum bangense	9.5–10		0	Xu et al. (1999)
Aeropyrum pernix	7	90–95	0	Sako et al. (1996); Morii et al. (1999)
Desulfurococcus mobilis	6	85	>75	Lanzotti et al. (1987)
Igniococcus islandicus fumarii	5.8	90	50	Huber et al. (2000)
Pyrolobus fumarii	5.5	106	95	Blochl et al. (1997)
Thermosphaera aggregans	6.5	85	95	Huber et al. (1998)
Pyrobaculum aerophilum	7	100	95	Trincone et al. (1992); Volkl et al. (1993)
P. islandicum	6	100	95	Trincone et al. (1992)
P. oguniense	6.3–7	92	95	Trincone et al. (1992)
P. organotrophum	6	100	95	Trincone et al. (1992)
Thermophilum pendens	5.5	88	95	Zillig et al. (1983)
Desulfurolobus ambivalens	2.5	80	100	Trincone et al. (1989)
Metallosphaera sedula	2.8	75	100	Itoh et al. (2001)
Nonthermophilic marine *Crenarchaeota*	~7	~25	>50	DeLong et al. (1998); Hoefs et al. (1997)

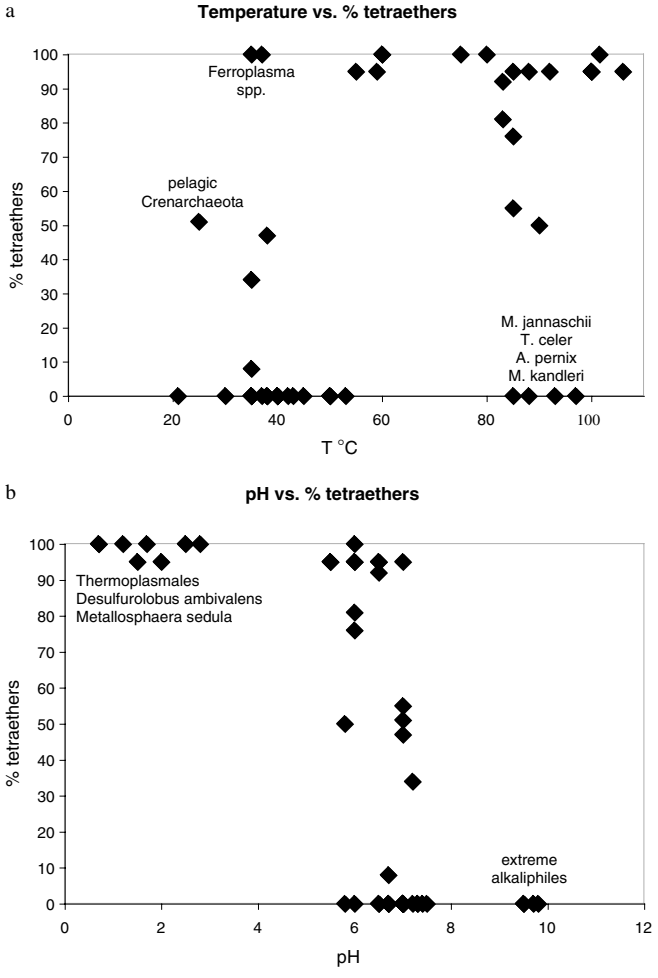

Fig. 6 a,b Membrane core lipid composition (diether vs tetraether) of archaeal isolates plotted against temperature optima (**a**) and pH optima (**b**). Percentages are $\pm 10\%$ for $n = 49$ isolates

for total lipid extracts of *F. acidarmanus* and *F. acidiphilum* are quite different (Figs. 4, 5), suggesting a different pattern of head group ornamentation between the two strains despite identical core lipids. In particular, *F. acidiphilum* membranes contain a significant fraction of lipids with lower m/z (e.g. $[M\text{-}H]^-$ $m/z = 1{,}447.1$) than those in *F. acidarmanus*, indicating lipids with lower molecular weights on average in *F. acidiphilum*. Membrane lipid head groups have important effects on the membrane potential, the mobility of lipids in the plane of the membrane, and other chemical properties that influence the permeability of the membrane to metal ions and to proton-transmitting water wires. The difference in head group structures may contribute to a difference in pH stability of the membranes and thus to the distinctive optimal growth pH of these related archaea.

Isoprene-derived, ether-linked lipids are among a small handful of physiological characteristics that establish the archaea as completely distinct from bacteria and eukaryotes. Their properties can be tuned to environmental conditions via variations in the saturation state, chain length, head group composition, number and placement of heptane, hexane or pentane rings and hydroxyl substituents, and perhaps most importantly, by fusion of opposing diether bilayers to form monolayer membrane regions or entire membrane monolayers. Tetraether-linked, monolayer membrane lipids are thought to be adaptations for maintaining membrane stability at high temperatures. However, several hyperthermophilic archaea have diether-only membranes (Table 1, Fig. 6a). The recent discovery of ubiquitous low-temperature marine Crenarchaeota having tetraether-dominated membranes is also inconsistent with this simple picture. New data presented here for nonthermophilic *Ferroplasma* isolates further weaken the correlation between core lipid chemistry and temperature. Across the archaeal domain, the temperature optimum is a poor predictor of membrane core lipid composition (Fig. 6a).

There is a more consistent relationship between membrane core lipid composition and pH optimum (Fig. 6b). Because protons are thought to leak through lipid membranes via transient water threads (Nagle and Morowitz 1978), fusion of lipid bilayers to form a monolayer should confer a selective advantage for acidophiles (van De Vossenberg et al. 1998). Combining the results for *Ferroplasma* spp with published data (Langworthy 1982; Schleper et al. 1995; Swain et al. 1997; Trincone et al. 1989; Trincone et al. 1992) indicates that tetraether-linked lipids comprise the majority of membrane lipids in all known acidophilic archaea (Fig. 6b). Likewise, all known extreme alkaliphiles have exclusively diether core lipids. Because all acidophilic archaea characterized to date produce extremely proton-impermeable tetraether membranes, we propose that a membrane monolayer is a key characteristic that enables archaea to live in acid.

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