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ORIGINAL PAPER

Polar lipid fatty acids, LPS-hydroxy fatty acids, and respiratory quinones of three *Geobacter* strains, and variation with electron acceptor

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Abstract The polar lipid fatty acids, lipopolysaccharide hydroxy-fatty acids, and respiratory quinones of *Geobacter metallireducens* str. GS-15, *Geobacter sulfurreducens* str. PCA, and *Geobacter bemidjiensis* str. Bem are reported. Also, the lipids of *G. metallireducens* were compared when grown with Fe^{3+} or nitrate as electron acceptors and *G. sulfurreducens* with Fe^{3+} or fumarate. In all experiments, the most abundant polar lipid fatty acids were 14:0, i15:0, 16:1ω7c, 16:1ω5c, and 16:0; lipopolysaccharide hydroxy-fatty acids were dominated by 3oh16:0, 3oh14:0, 9oh16:0, and 10oh16:0; and menaquinone-8 was the most abundant respiratory quinone. Some variation in lipid profiles with strain were observed, but not with electron acceptor.

Keywords *Geobacter* · Polar lipid fatty acids · Lipopolysaccharide fatty acids · Respiratory quinines · Lipid analysis

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Abbreviations

LPS ohFA	Lipopolysaccharide hydroxy-fatty acids
PLFA	Polar lipid fatty acids
MK	Menaquinone

Introduction

Geobacter is a versatile Deltaproteobacterium that can utilize Fe^{3+}, Mn^{4+}, U^{6+} [18], or oxygen [14] as electron acceptors which has important roles in geochemical cycling [18], bioremediation [25, 32], and electrical generation [30]. In support of ecological studies using ^{13}C-labeled substrate incorporation into lipid biomarkers of *Geobacter*, and general biochemical studies, the polar lipid fatty acids (PLFA), lipopolysaccharide hydroxy-fatty acids (LPS ohFA), and respiratory quinones of three strains of *Geobacter* were determined, and on two of the strains with different electron acceptors.

Materials and methods

Strains and culturing

Geobacter metallireducens str. GS-15 [18], *Geobacter sulfurreducens* str. PCA [4], and *Geobacter bemidjiensis* str. Bem [23] were cultured in freshwater medium [19] with 55 mM Fe^{3+} citrate as electron acceptor and 10 mM acetate as electron donor. *G. metallireducens* was also cultured in the same medium with 5 mM nitrate replacing Fe^{3+} citrate as electron acceptor, and *G. sulfureducens* was cultured in NB medium with 40 mM fumarate as electron acceptor and 15 mM acetate as electron donor [17]. All culturing was in batch at 30°C under standard anaerobic conditions [1, 26].

Cells were harvested by centrifugation at 3,200*g* for 20 min and frozen at −20°F until analysis.

Polar lipid fatty acid analysis

Dried cell material was extracted using the method of Bligh and Dyer [3] as modified [29]. Complete extraction was ensured by sonication for 2 min and extraction for 4 h before the separation of the aqueous and organic phases. The total lipid extract was then fractionated into neutral lipid, glycolipid, and phospholipid by elution with chloroform, acetone, and methanol, respectively, using 500 mg silicic acid columns [10]. Neutral lipids were reserved for quinone analysis. The polar lipids were transesterified to the fatty acid methyl esters using a mild alkaline methanolysis [10].

An Agilent 6890 series gas chromatograph with a 50 m nonpolar column (0.2 mm I.D., 0.11 μm film thickness) was used for gas chromatographic analysis. The temperature program was initial temperature = 100°C, 10°C/min to 150°C, 150°C for 1 min, 3°C/min to 282°C, and hold at 282°C for 5 min. The injector and detector temperatures were 270 and 290°C, respectively. Peak quantitation and mass identification was performed using an Agilent 5973 mass-selective detector. Monounsaturates were identified by derivatization with dimethyldisulfide and gas chromatography/mass spectroscopy as described [24].

Lipopolysaccharide hydroxy-fatty acid analysis

Dried cell material was treated with a strong acid methanolysis (chloroform/methanol/hydrochloric acid 10:1:1 at 100°C for 1 h) to liberate the fatty acids as their methyl esters [12]. The hydroxy moieties were converted to trimethylsilyl ethers and analyzed by the same instrumentation and methods as the polar lipid fatty acids. Only the hydroxy-fatty acids were reported.

Respiratory quinone analysis

The respiratory quinones in the neutral lipid fraction of the total lipid extract were determined by atmospheric pressure chemical ionization tandem mass spectrometry as described [9].

Results

Polar Lipid Fatty Acids

The PLFA profiles (Table 1) of the *Geobacter* strains analyzed were very similar to each other, with 14:0, i15:0, 16:1ω7c, 16:1ω5c, and 16:0 more than 1% of the total for all strains under all conditions. 18:1ω7c was also more than 1% in some samples. The biggest differences between strains were in i15:0 and 18:1ω7c (*G. metallireducens* > *G. sulfurreducens* > *G. bemidjiensis*). The effect of electron acceptor on the PLFA profiles were less than the differences between the strains. *G. metallireducens* with nitrate as the electron acceptor and *G. sulfurreducens* with fumarate had slightly higher proportions of 16:1ω7c and less 16:0 compared to with ferric iron as the electron acceptor.

Lipopolysaccharide hydroxy-fatty acids

The LPS oh-FA (Table 1) were more variable between strains and electron acceptors than the PLFA. *G. metallireducens* was distinguished by having over four times more 3oh16:0 than 3oh14:0, and significant amounts of 9oh16:0 and 10oh16:0 (10–15%), while *G. sulfurreducens* and *G. bemidjiensis* had more similar proportions of 3oh16:0 and 3oh14:0 and much less 9oh16:0 and 10oh16:0 (5–0.4%). As was found for the PLFA, the variation between strains was greater than the variation with electron acceptor.

Respiratory quinones

The respiratory quinone profiles (Table 1) were dominated by menaquinone-8 (MK-8), which was more than 85% of the total quinones for all strains under all conditions. Ubiquinones were not detected above background. Overall, the quinone profiles were very similar to each other between strains and treatments.

Discussion

The PLFA and LPS ohFA composition of *G. metallireducens* under iron- and nitrate-reducing conditions has been determined [18]. While the overall lipid profiles were very similar, the shifts in the profiles with change in electron acceptor were different. In this work (Table 1) all of the major PLFA (>1%) decreased except for 16:1ω7c when the electron acceptor was changed from ferric iron to nitrate. However, in the experiment performed over 10 years earlier, 14:0 and i15:0 increased. Similarly for the LPS oh-FA, in this work 3oh14:0, 9oh16:0, and 10oh16:0 increased, while in [18] 3oh14:0 and 3oh15:0 increased. *G. metallireducens* grown with Fe^{3+} citrate had similar overall profiles, except that 18:1ω7c was 29% [36], up from 1.1 to 3.7% in this study and [18]. This suggests that the PLFA and LPS oh-FA of *G. metallireducens* are less affected by the change in electron acceptor, more by details of culturing conditions. Growth phase, ionic strength, temperature, toxic compounds, and carbon source have all been shown to shift bacterial fatty acid profiles.

Table 1 Polar lipid fatty acids, lipopolysaccharide hydroxy-fatty acids, and respiratory quinones of three strains of *Geobacter* with different electron acceptors, as mole percents, average and standard deviation ($n = 3$)

Strain	*Geobacter metallireducens*, Avg (SD)		*Geobacter sulfurreducens*, Avg (SD)		*G. bemidjiensis*, Avg (SD)
e^- acceptor	Fe^{3+}	Nitrate	Fe^{3+}	Fumarate	Fe^{3+}
Polar lipid fatty acids					
14:1ω7c D	0.04 (0.03)	0.03 (0.05)	0.20 (0.03)	0.28 (0.01)	0.38 (0.08)
14:1ω7t	0.05 (0.04)	0.03 (0.05)	0.07 (0.02)	0.08 (0.00)	0.09 (0.02)
14:1ω5c					0.16 (0.05)
14:0	**6.39** (0.86)	**6.23** (5.10)	**9.19** (0.54)	**8.49** (0.14)	**7.67** (0.34)
i15:1ω7c	0.05 (0.04)	0.02 (0.03)	0.10 (0.02)	0.11 (0.01)	
i15:0	**9.88** (0.27)	**9.66** (2.26)	**6.31** (0.40)	**5.32** (0.11)	**1.07** (0.04)
a15:0	0.27 (0.05)	0.05 (0.09)	0.54 (0.06)	0.27 (0.01)	0.14 (0.04)
15:1				0.01 (0.01)	0.13 (0.04)
15:0			0.61 (0.74)	0.20 (0.02)	0.14 (0.03)
16:1ω7c D	**48.44** (1.08)	**57.40** (3.36)	**35.87** (0.55)	**38.30** (0.20)	**46.02** (0.79)
16:1ω7t D	0.74 (0.12)	0.91 (0.23)	0.41 (0.09)	0.39 (0.05)	0.70 (0.02)
16:1ω5c D	**1.65** (0.10)	**1.49** (0.36)	**1.11** (0.07)	**1.41** (0.03)	**2.22** (0.01)
16:0	**29.27** (0.37)	**22.66** (3.76)	**43.52** (0.63)	**42.69** (0.19)	**40.56** (0.78)
i17:1ω7c D	0.13 (0.02)	0.09 (0.08)	0.14 (0.04)	0.11 (0.02)	
10Me16:0	0.46 (0.06)	0.19 (0.07)	0.18 (0.06)	0.12 (0.06)	0.06 (0.06)
i17:0	0.25 (0.04)	0.08 (0.07)	0.08 (0.02)	0.07 (0.01)	
18:1ω7c D	**2.00** (0.19)	0.94 (0.34)	**1.13** (0.10)	**1.24** (0.03)	0.48 (0.07)
18:1ω5c	0.09 (0.03)		0.05 (0.00)	0.08 (0.01)	
18:0	0.28 (0.06)	0.19 (0.06)	0.30 (0.03)	0.52 (0.01)	0.14 (0.01)
br19:1			0.06 (0.00)	0.09 (0.01)	
Lipopolysaccharide hydroxy-fatty acids					
3oh12:0			0.7 (1.1)	1.3 (1.3)	0.2 (0.1)
3oh13:0					0.1 (0.1)
3oh14:0	**11.1** (1.9)	**13.4** (2.4)	**30.0** (4.1)	**46.9** (8.5)	**47.8** (1.9)
3oh-i15:0			4.4 (0.4)	7.6 (1.4)	7.1 (0.1)
3oh-a15:0				1.3 (2.2)	0.4 (0.1)
3oh15:0	6.7 (5.8)		1.3 (2.3)		0.6 (0.1)
3oh16:0	**62.3** (6.1)	**55.9** (10.7)	**53.5** (4.1)	**38.2** (5.9)	**42.8** (1.8)
9oh16:0	9.0 (1.6)	**14.1** (5.7)	4.5 (0.2)	2.2 (1.9)	0.4 (0.3)
10oh16:0	**11.0** (1.7)	**16.6** (6.4)	5.5 (0.3)	2.5 (2.3)	0.6 (0.0)
Respiratory quinones					
MK-4	3.39 (0.15)	3.28 (0.21)	2.77 (0.13)	3.39 (0.55)	3.07 (0.07)
MK-5	1.52 (0.11)	1.50 (0.06)	1.36 (0.02)	1.41 (0.09)	1.48 (0.06)
MK-6	4.73 (0.06)	4.68 (0.08)	4.35 (0.09)	5.30 (1.00)	4.61 (0.04)
MK-7	0.68 (0.04)	0.93 (0.06)	0.66 (0.02)	0.77 (0.17)	1.21 (0.01)
MK-8	**85.18** (0.36)	**86.86** (0.16)	**87.17** (0.16)	**86.69** (1.67)	**87.21** (0.25)
MK-9	4.45 (0.33)	2.73 (0.18)	3.68 (0.14)	2.44 (0.14)	2.41 (0.17)

Major components (fatty acids > 1%, LPS oh-FA and MK > 10%) are in bold. Fatty acids marked with a "D" were validated by DMDS derivatization. Fatty acids not greater than 0.1% in any analysis were not reported, including i14:0, 3oh14:0, i16:0, a16:0, 11Me16:0, Cy17:0, 17:0, br18:1, 3oh16:0, and 18:1ω7t

The PLFA profiles distinguished *G. metallireducens*, *sulfurreducens*, and *bemidjiensis* in pure culture under controlled conditions, but could not be used to determine which organism is present in the complex microbial community of an environmental sample. It will also be difficult to distinguish some Deltaproteobacterial sulfate-reducers

from *Geobacter* in environmental samples by PLFA analysis, unless *Geobacter* is a high proportion of the community. *Desulfuromonas acetoxydans* [6], a co-member of the Desulfuromonadales with *Geobacter*, has a very similar profile to the *Geobacters* discussed above. Desulfovibrionales have more iso- and anteiso-branched 15- and 17-carbon fatty acids and i17:1ω7c [7, 8, 15, 34]. Desulfobacteraceae have more 10Me16:0 and Cy17:0 [6, 15, 34]. However, in the case of subsurface biostimulation of U(VI) immobilization by *Geobacter*, the high cell numbers of *Geobacter* in treated sediments allows monitoring its population by PLFA analysis [22].

The LPS ohFA could be used to detect the growth of *Geobacter* in the subsurface during bioremediation. The strains analyzed had unusually high levels of 3oh16:0 (Table 1), and the unusual fatty acids 9oh16:0 and 10oh16:0. The literature on LPS FA is incomplete, especially for environmental isolates, but a study of 6 strains of *Desulfovibrio* found 3oh-i15:0 or 3oh-i17:0 as the most abundant LPSFA [7]. 3oh16:0 was only 5.8–16.6% in these strains. A saturated subsurface sediment sample had 16.9% 3oh16:0 in the LPSFA, and no 3oh16:0 in any of the 4 Gammaproteobacterial strains tested [27]. Since LPSFA are not degraded as quickly as PLFA upon cell death, sediment samples can have much more LPSFA than can be accounted for by the viable microbial biomass, representing "fossil" bacteria (Hedrick and White, unpublished). Therefore, groundwater or Bio-Trap® samples [28] would be more appropriate for this analysis.

Menaquinone-8 accounted for over 85% of the menaquinones and no ubiquinones were detected for all three strains and two redox acceptor experiments. MK-8 has been detected before in *G. sulfurreducens* [21]. The high proportion of this single respiratory quinone in *Geobacter* makes it a possible candidate as a biomarker. Anaerobic Gammaproteobacteria and Bacteroidetes often have MK-8, [5], but the sulfate-reducers usually do not. MK-6 has been reported from *Desulfovibrio desulfuricans*, *Desulfovibrio gigas*, and *Desulfovibrio vulgaris* [11, 20, 35]; MK-7 in *Desulfovermiculus halophilus* [2]; MK-5(H2) in *Desulfobulbus rhabdoformis* [13]; MK-7(H2) in *Desulfovibrio magneticus* [31]; no menaquinones were detected in *Desulfomonile tiedjei* DCB-1 [16]; and only *Desulfopila aestuarii* had MK-8 [33].

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