Effects of Aerobic Growth on the Fatty Acid and Hydrocarbon Compositions of 
*Geobacter bemidjiensis* BemT

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Abstract: *Geobacter* spp., regarded as strict anaerobes, have been reported to grow under aerobic conditions. To elucidate the role of fatty acids in aerobiosis of *Geobacter* spp., we studied the effect of aerobiosis on fatty acid composition and turnover in *G. bemidjiensis* BemT. *G. bemidjiensis* BemT was grown under the following different culture conditions: anaerobic culture for 4 days (type 1) and type 1 culture followed by 2-day anaerobic (type 2) or aerobic culture (anaerobic-to-aerobic shift; type 3). The mean cell weight of the type 3 culture was approximately 2.5-fold greater than that of type 1 and 2 cultures. The fatty acid methyl ester and hydrocarbon fraction contained hexadecanoic (16:0), 9-cis-hexadecenoic [16:1(9c)], tetradecanoic (14:0), tetradecenoic [14:1(7c)] acids, hentriacontanonaene, and hopanoids, but not long-chain polyunsaturated fatty acids. The type 3 culture contained higher levels of 14:0 and 14:1(7c) and lower levels of 16:0 and 16:1(9c) compared with type 1 and 2 cultures. The weight ratio of extracted lipid per dry cell was lower in the type 3 culture than in the type 1 and 2 cultures. We concluded that anaerobically-grown *G. bemidjiensis* BemT followed by aerobiosis were enhanced in growth, fatty acid turnover, and *de novo* fatty acid synthesis.

Key words: *Geobacter*, anaerobic growth, aerobic growth, fatty acid, hydrocarbon

1 INTRODUCTION

Some limited groups of bacteria produce distinct unsaturated fatty acids with chain lengths longer than 20 carbons and at least four double bonds, using the Pfa enzyme complex, PUFA synthase, which is specifically found in bacteria, encoded by the *pfa* genes1,2. Long-chain PUFAs (LC-PUFAs) include arachidonic acid (ARA, 20:4 n-6), eicosa-pentaenoic acid (EPA, 20:5 n-3), and docosahexaenoic acid (DHA, 22:6 n-3)3. These LC-PUFAs have an antioxidative function in bacteria4–8.

*Geobacter* species are strict anaerobic bacteria that predominate in subsurface environments9. The whole genome sequences of various *Geobacter* species, including the strains *G. sulfurreducens PCA*10, *G. bemidjiensis* BemT, *G. metallireducens*, and *G. lovleyi* SZ11 offer insights into their phylogenetic and physiological traits10–13. For example, analysis of the sequence of the genome of *G. sulfurreducens* PCA10 revealed the presence of genes encoding various oxidation-related enzyme homologs, including the high-oxygen-affinity cytochrome *d*-ubiquinol terminal oxidase and the low-oxygen-affinity cytochrome *c* oxidase, indicating that this bacterium uses molecular oxygen as a terminal electron acceptor12. Indeed, subsequent studies support this hypothesis13. The genome of *G. bemidjiensis* BemT includes similar gene homologs that are involved in aerobic respiration, indicating its capacity to grow under...
aerobic conditions\textsuperscript{10}. \textit{G. bemidjiensis} DSM 16622\textsuperscript{T} (designated as strain Bem\textsuperscript{T} hereafter) was initially isolated from sediments of an aquifer in Bemidji, Minnesota, USA, and is classified as an obligate anaerobe because of its growth under the strict anaerobic conditions used for culturing microorganisms that reduce Fe(III)\textsuperscript{9}. Although characterization of the lipid components of this bacterium is limited, hexadecanoic (16:0) and \((\Delta 9\)-cis hexadecenoic [16:1(9c)]\) acids, as well as 3-hydroxy tetradecanoic (3-OH 14:0) and 3-hydroxy hexadecanoic (3-OH 16:0) acids, which are major polar lipids and lipopolysaccharide fatty acids, respectively, are common fatty acid components of various \textit{Geobacter} species\textsuperscript{15}. Furthermore, certain \textit{Geobacter} species produce hopanoid lipid hydrocarbons (HCs) that have not been quantified in \textit{G. bemidjiensis} Bem\textsuperscript{T}\textsuperscript{16}. The genome of \textit{G. bemidjiensis} Bem\textsuperscript{7} includes the tandem clusters \textit{pfaD}	extsuperscript{{\(\frac{\text{II}}{3}\)}}, \textit{pfaA-pfaB/C} and \textit{pfaE} and \textit{oleA}, \textit{oleB}, \textit{oleC}, and \textit{oleD} that encode components of the biosynthetic pathway of LC-PUFAs such as DHA and EPA, and the very LC polyunsaturated HC (3Z,6Z,9Z,12Z,15Z,19Z,22Z,25Z,28Z)-hentriaconta-3,6,9,12,15,19,22,25,28-nonaene (C31:9), respectively\textsuperscript{17}. Although \textit{G. bemidjiensis} Bem\textsuperscript{T} produces C31:9\textsuperscript{19}, its ability to synthesize LC-PUFAs is unknown. Bacterial LC-PUFAs possess multiple bis-allylic hydrogens, which act unexpectedly as antioxidants, and are highly susceptible to oxidation\textsuperscript{1, 5, 7, 19, 20}. We hypothesized that \textit{G. bemidjiensis} Bem\textsuperscript{T} might synthesize LC-PUFAs or LC-HCs, both of which are assumed to function as antioxidants, to protect the cells against oxidative stresses in the presence of oxygen, and accordingly change the lipid composition of the cellular membrane.

The objective of this study was to understand how aerobiosis affects the fatty acid composition and turnover in \textit{G. bemidjiensis} Bem\textsuperscript{T} through anaerobic-to-aerobic shifts. We analyzed fatty acid and HC components in this strain to determine the relationships between its growth characteristics and lipid and fatty acid compositions.

2 EXPERIMENTAL

2.1 Bacterial strains and culture conditions

\textit{G. bemidjiensis} DSM 16622\textsuperscript{T} (strain Bem\textsuperscript{T}) and \textit{G. metallireducens} DSM 7210\textsuperscript{T} (strain GS-15\textsuperscript{T}) were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Germany, and were cultivated in strict anaerobic conditions in DSMZ 579 \textit{Geobacter} medium supplemented with L-cysteine-HCl as a reducing agent at a final concentration of 0.05\% (w/v). Cells were cultivated in 1200-mL bottles with butyl rubber stoppers in anaerobic and static conditions, according to previously described techniques\textsuperscript{21}. Culture bottles contained O\textsubscript{2}-free N\textsubscript{2}:CO\textsubscript{2} (80:20, v/v) in the headspace. Anaerobic starter cultures of \textit{G. bemidjiensis} Bem\textsuperscript{T} were cultivated in 100 mL of the same medium in 110-mL bottles under static conditions for 14 days at 30°C, and were then transferred into nine 1200-mL bottles. Subsequently, \textit{G. bemidjiensis} Bem\textsuperscript{T} was cultivated in triplicate under the conditions shown in Fig. 1. Type 1 and 2 cultures were anaerobically incubated for 4 and 6 days, respectively, and type 3 cultures were anaerobically incubated for 4 days and then aerobically for 2 days. After anaerobic culture, type 3 cultures were transferred to sterilized 3000-mL flasks with a porous silicon-stopper and aerobically shaken at 50 rpm for 2 days at 30°C (anaerobic-to-aerobic shift).

Cells were harvested by centrifugation at 7,000 x g for 30 min, washed twice with 5 mM NaCl, freeze-dried, weighed, and stored at −80°C.

\textit{G. metallireducens} DSM 7210\textsuperscript{T} served as the reference strain for comparisons of hopanoid lipid contents and was anaerobically cultivated as described above. \textit{Shevannella oshoroensis} strain osh08\textsuperscript{17} and \textit{Moritella marina} ATCC 15381\textsuperscript{T} (strain MP-1\textsuperscript{T}), both of which produce LC-PUFA and C31:9, were cultivated in Marine Broth 2216 (Difco) as described previously\textsuperscript{22–25}. EPA, DHA, and C31:9 from these bacteria were used as reference compounds.

2.2 Measurement of dissolved oxygen concentrations of cultures

The dissolved oxygen (DO) concentrations of \textit{G. bemidjiensis} Bem\textsuperscript{T} cultures were measured immediately before
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3 RESULTS and DISCUSSION
3.1 Anaerobic and aerobic growth characteristics of G. bemidjiensis BemT

The DO concentrations in the media that were measured immediately before harvesting cells were as follows: 0.057 ± 0.028 mg O2/L, 0.028 ± 0.011 mg O2/L, and 4.015 ± 0.007 mg O2/L in type 1, 2, and 3 cultures, respectively (Table 1), compared with 0.035 ± 0.021 mg O2/L in uninoculated media. These results show that type 3 cultures were more aerobic than types 1 and 2, and uninoculated media. However, when compared with the value in uninoculated media, the differences among type 1, 2, and uninoculated media were not significant. The DO concentration in the culture media might be far below the detection limit of the DO meter used in this study. We thus concluded that type 1, 2, and uninoculated media maintained sufficient anaerobic conditions.

The mean dry cell weight was greater in type 3 cultures (141.7 ± 21.7 mg) than in type 1 and 2 cultures (56.3 ± 0.9 mg and 55.0 ± 0.0 mg, respectively) (Table 1). The growth of G. bemidjiensis BemT was also evaluated according to total extractable lipids from dried cells. Lipid weights (fatty acid and HC fractions) from type 1, 2, and 3 cultures were 5.0 ± 1.2 mg, 6.0 ± 0.0 mg, and 9.0 ± 2.9 mg, respectively (Table 1), indicating that the growth of G. bemidjiensis BemT plateaued by day 4 under anaerobic conditions, but continued for 2 days under aerobic conditions. In contrast, the aerobic growth of anaerobically cultivated G. bemidjiensis BemT starter cultures did not occur after direct inoculation into the same medium with immediate shaking under aerobic conditions. Since the ratios of lipid to dry cell weights were 0.09, 0.11, and 0.06 from type 1, 2, and 3 cultures, respectively (Table 1), the aerobic growth of G. bemidjiensis BemT may reflect consumption of stored lipids that accumulated under anaerobic conditions.

3.2 Fatty acid and hydrocarbon components

The GC profile of the FAME plus HC fraction derived from dry cells after type 1 culture is shown in Fig. 2. This GC profile is almost identical to that of the FAME plus HC fraction derived from the total lipids of type 1 cultures. The 14:0, 16:0, and 16:1 (9c) fatty acids were the major saturated and monounsaturated fatty acids. Dodecanoic (12:0), iso-pentadecanoic (iso-15:0), and octadecanoic (18:0) acids were minor components, and hydroxy fatty acids such as 3-hydroxy tetradecanoic (3-OH 14:0), pentadecanoic (3-OH 15:0), and hexadecanoic (3-OH 16:0) acids were detected. With the exception of 12:0, all fatty acid components are present in G. bemidjiensis BemT and

2.3 Extraction of total lipids

Total lipids were extracted from 20–30 mg of dry cells, using chloroform and methanol(26). Lipids were dried using a stream of nitrogen gas, weighed, and dissolved to 50 mg/mL in a mixture of chloroform and methanol (2:1, v/v) containing 0.01% (w/v) dibutylhydroxytoluene. These samples were stored at −30°C until use.

2.4 Preparation and analysis of fatty acid methyl esters and hydrocarbons

Dry cells (2–5 mg) and total lipids (approximately 2 mg) were subjected to methanolysis, using 2 mL of 10% (v/v) acetyl chloride in methanol at 100°C for 1 h. Henecicosanoic acid (100 µg, 21:0; Sigma-Aldrich, Tokyo, Japan) was added, and fatty acid methyl esters (FAMEs) were extracted three times using hexane. FAME fractions included HCs were designated as FAME plus HC fractions(27). FAME was separated from HCs by one-dimensional thin-layer chromatography (TLC) with silica gel plates (type G60; Merck, Darmstadt, Germany) and a solvent system comprising hexane, diethyl ether, and acetic acid, 90:10:1 (v/v/v), with slight modifications, as described previously(27). The primuline solution was sprayed with an all-glass atomizer, and the plates were then visualized under UV light (340 nm). Spots with different Rf values, which contained FAMEs or HCs, were recovered from the silica gel using hexane, after scraping them off the plates.

Fatty acids and HCs were identified by comparing retention times with those of authentic standards or fatty acids and HCs derived from the reference strains. For this purpose, we used a gas chromatograph (GC) (Model GC-353B, GL Sciences, Tokyo, Japan) equipped with a polar capillary column (type TC-70, 30-m long, 0.25-mm I.D., 0.25-µm film thickness; GL Sciences Inc, Tokyo) and a flame ionization detector with nitrogen as the carrier gas. Dodecanoic (12:0), iso-pentadecanoic (iso-15:0), and octadecanoic (18:0) acids were major components, and hydroxy fatty acids such as 3-hydroxy tetradecanoic (3-OH 14:0), pentadecanoic (3-OH 15:0), and hexadecanoic (3-OH 16:0) acids were detected. With the exception of 12:0, all fatty acid components are present in G. bemidjiensis BemT and

2.5 Statistical analyses

All experiments were performed in triplicate, and the mean and standard error of the mean (SEM) were calculated using Excel software (Microsoft). A p-value of less than 0.05 was considered statistically significant.
Table 1 Effects of anaerobic-to-aerobic shift on the growth and major fatty acid and hydrocarbon compositions of G. bemidjiensis Bem\textsuperscript{T}.

<table>
<thead>
<tr>
<th>Culture type\textsuperscript{a}</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved O\textsubscript{2} (mg O\textsubscript{2}/L)</td>
<td>0.057 ± 0.028</td>
<td>0.028 ± 0.011</td>
<td>4.015 ± 0.007</td>
</tr>
<tr>
<td>Dry cell (mg/L)</td>
<td>56.3 ± 0.9</td>
<td>55.0 ± 0</td>
<td>141.7 ± 21.7</td>
</tr>
<tr>
<td>Total lipid (mg/L)</td>
<td>5.0 ± 1.2</td>
<td>6.0 ± 0</td>
<td>9.0 ± 2.9</td>
</tr>
<tr>
<td>Total lipid/dry cell</td>
<td>0.09</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>Fatty acid + hydrocarbon (mg/L)</td>
<td>2.0 ± 0</td>
<td>3.0 ± 0</td>
<td>5.0 ± 1.0</td>
</tr>
<tr>
<td>Hydrocarbon/Fatty acid + hydrocarbon</td>
<td>3.6 ± 2.6</td>
<td>1.2 ± 0.6</td>
<td>1.8 ± 0.7</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Culture type: Type 1 culture, normal anaerobic culture for 4 days; type 2 culture, type 1 culture followed by anaerobic culture for 2 days; and type 3 culture, type 1 culture followed by aerobic culture for 2 days.

\textsuperscript{b}Others included fatty acids 12:0, iso-15:0, 15:0, 18:1, 3-OH 14:0, 3-OH 15:0, 3-OH 16:0, and unidentified fatty acids and hydrocarbons were <0.5%.

Fatty acids are abbreviated as follows: X:Y (\(\Delta Z\)) t, where X and Y are the numbers of carbon atoms and double bonds, respectively, and Z indicates the position of the double bond with a cis (c) or trans (t) configuration from the carboxyl end; iso-15:0, iso-branched 15:0, 3-OH, 3-hydroxy; C31:9, all-

<table>
<thead>
<tr>
<th>Fatty acid and hydrocarbon composition (% of the total)</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>9.5 ± 0.4</td>
<td>8.9 ± 0.4</td>
<td>14.5 ± 0.7</td>
</tr>
<tr>
<td>14:1(7c)</td>
<td>1.7 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>16:0</td>
<td>32.3 ± 0.7</td>
<td>32.1 ± 0.1</td>
<td>28.6 ± 0.3</td>
</tr>
<tr>
<td>16:1(9c)</td>
<td>49.9 ± 1.0</td>
<td>50.6 ± 0.1</td>
<td>46.3 ± 1.1</td>
</tr>
<tr>
<td>16:1(11c)</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.1</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>18:1(11c)</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>C31:9</td>
<td>0.6 ± 0.2</td>
<td>1.2 ± 0.5</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Hop-17(21)-ene</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Others\textsuperscript{b}</td>
<td>2.8 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>4.1 ± 1.0</td>
</tr>
<tr>
<td>C14 fatty acids</td>
<td>11.2</td>
<td>10.5</td>
<td>16.8</td>
</tr>
<tr>
<td>C16 fatty acids</td>
<td>82.2</td>
<td>82.7</td>
<td>74.9</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Culture type: Type 1 culture, normal anaerobic culture for 4 days; type 2 culture, type 1 culture followed by anaerobic culture for 2 days; and type 3 culture, type 1 culture followed by aerobic culture for 2 days.

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Fatty acids are abbreviated as follows: X:Y (\(\Delta Z\)) t, where X and Y are the numbers of carbon atoms and double bonds, respectively, and Z indicates the position of the double bond with a cis (c) or trans (t) configuration from the carboxyl end; iso-15:0, iso-branched 15:0, 3-OH, 3-hydroxy; C31:9, all-

other Geobacter species\textsuperscript{25, 30, 31}. LC-PUFAs were not detected in type 1, 2, and 3 cultures (Fig. 2, details below).

Various HC species were detected as minor components of the FAME plus HC fraction of G. bemidjiensis Bem\textsuperscript{T} (Fig. 2). HCs with mass fragments at \(m/z\) 57, 71, 83(85), 97(99), and 113 were considered as \(n\)-alkanes or \(n\)-monoalkenes\textsuperscript{22}. However, with the exception of \(n\)-hexadecane (C16:0; HC\textsubscript{1} in Fig. 2 and S1), most normal HCs (HC\textsubscript{2}–HC\textsubscript{6} in Fig. 2) were not identified because of undetectable levels of their parent ions. Since the retention time of authentic tetracosane (C24:0) was approximately 20.2 min (Fig. 2), the chain length of alkanes (HC\textsubscript{0} to HC\textsubscript{6}) was probably medium or long. Among HCs, the peak retention time at 36.7 min (HC\textsubscript{3}) was identified as C31:9, which was the same as that of C31:9 from the two C31:9-producing bacteria S. oshoroensis osh08\textsuperscript{T} and M. marina MP-1\textsuperscript{T} (data not shown) and from the corresponding mass-fragment profile characteristics of polyolefinic carbon chains (Fig. 3a and 3b).

The detection of C31:9 in G. bemidjiensis Bem\textsuperscript{T} is consistent with the study reported by Sukovich et al.\textsuperscript{38}, and strongly indicates the presence of LC-PUFAs in this bacterium because the synthesis of one mole of C31:9 requires two moles of (4Z,7Z,10Z,13Z)-hexadec-4,7,10,13-tetraenoic acid (16:4) as a substrate, which is a product (and a precursor of EPA and DHA) of PUFA synthase\textsuperscript{33}. However, no clear peaks corresponding to EPA or DHA were detected (Fig. 2). To detect LC-PUFAs in type 1 cultures, total ion chromatograms with 25–40-min retention times were rescanned using a combination of ions at \(m/z\) 67, 79, 91, and
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105 (Fig. 3a), which are diagnostic fragment ions of straight chain polyolefinic compounds\(^2\). At least four very faint peaks (unknown peaks, designated as U\(_1\)-U\(_4\)) had fragment profiles with ions at \(m/z\) 67, 79, 91, and 105, respectively. The retention times of the components U\(_1\) and U\(_2\) were similar to those of EPA and DHA, respectively, but were not the same. These four peaks were therefore considered polyolefinic HCs with shorter chains, fewer unsaturated chains than C\(_{31}:9\), or both. The GC-MS fragment profile of peak U\(_2\) (Fig. 3c) indicates that the PUFA synthase synthesizes 16:4, which are much shorter PUFAs, and that two molecules of each species are subsequently condensed by the Ole enzyme complex, which is encoded in the \(ole\) gene cluster, to produce various polyolefinic HCs, including C\(_{31}:9\), along with the very minor peaks U\(_1\), U\(_3\), and U\(_4\). Among the four peaks, only U\(_3\) was detected in type 2 and type 3 cultures (data not shown). One possible explanation for why \(G.\) bemidjienis\(s\) Bem\(^7\) does not produce LC-PUFAs would be that as soon as PUFAs are synthesized by the Pfa enzyme complex, they may be utilized as substrates for Ole proteins, which produce C\(_{31}:9\) through the head-to-head fatty acid condensation mechanism\(^1\).

The peak components HC\(_8\), HC\(_9\), and HC\(_{10}\) detected after C\(_{31}:9\) were HCs, which were recovered with C\(_{31}:9\) in a HC fraction from TLC analyses (data not shown). HC\(_8\) was identified as hop-17\(\_21\)-ene, which is hopanoid with one double bond with specific mass fragment ions at \(m/z\) 121, 161, 191, 231, and 367 and a molecular ion at \(m/z\) 410 (Table 2, Fig. 4b). These ions are consistent with those of hop-17\(\_21\)-ene retrieved from the library data (Table 2). They may reflect the presence of the \(G.\) bemidjienis\(s\) Bem\(^7\) gene encoding squalene cyclase domain-containing protein (YP\_002140151.1). Although hopene (as hop-21-ene) is present in \(G.\) metallireducens DSM 7210\(^7\) and \(G.\) sulfurreducens DMS 12127\(^T\), the molecular fragment ion profile of hop-17\(\_21\)-ene was slightly different from those of hop-21-ene (Table 2). Moreover, the significant fragment ion at \(m/z\) 231 of hop-17\(\_21\)-ene was detected in \(G.\) bemidjienis\(s\) Bem\(^7\) (Table 2, Fig. 4b), and \(G.\) metallireducens DSM 7210\(^7\) (Table 2). However, no further details of the fragment ion at \(m/z\) 231 of hop-21-ene were reported\(^8\). The peak of the unidentified HC\(_8\) species was detected as a shoulder of the HC\(_9\) peak (Figs. 2 and 4a), and the peak of the HC\(_{10}\) species included a mixture of at least three hopanoids (Fig. 4), reflecting the presence of a diagnostic

\[\text{Fig. 2} \quad \text{Electron impact gas chromatogram of the fatty acid plus hydrocarbon (HC) fraction derived from dried } \text{G. bemidjienis \(Bem^7\) cells (type 1 culture) grown in anaerobic conditions for 4 days at 30°C. HC\(_n\) is an identified or unidentified HC. Internal standard (I.S.) heneicosanoic acid (21:0). The open arrow indicates the retention time of tetracosane (C24:0).} \]

\[\text{Fig. 3} \quad \text{Electron impact gas chromatogram and mass fragment profile, scanned using a combination of ions at \(m/z\) 67, 79, 91, and 105. The gas chromatogram (a) is a part of that shown in Fig. 2. The mass fragment profiles of C31:9 and the peak U\(_2\) component are shown in (b) and (c), respectively. Peaks U\(_1\), U\(_3\), and U\(_4\) had the same fragment profiles as the U\(_2\) peak and C31:9.} \]

\[\text{Fig. 4} \quad \text{Electron impact gas chromatogram and mass fragment profile, scanned using a combination of ions at \(m/z\) 67, 79, 91, and 105. The gas chromatogram (a) is a part of that shown in Fig. 2. The mass fragment profiles of C31:9 and the peak U\(_2\) component are shown in (b) and (c), respectively. Peaks U\(_1\), U\(_3\), and U\(_4\) had the same fragment profiles as the U\(_2\) peak and C31:9.} \]
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hopanoid mass fragment ion at m/z 191 generated from these compounds (data not shown). However, these hopanoid-like compounds (HC₁₀) were not identified in the present study.

3.3 Fatty acid and hydrocarbon compositions under different culture conditions

The present data provide evidence that *G. bemidjiensis* Bemᵀ grew in aerobic conditions (Table 1), but do not prove that *G. bemidjiensis* Bemᵀ utilizes molecular oxygen as an electron acceptor. However, the DO concentration (4.015 ± 0.007 mg O₂/L) of type 3 culture strongly indicates mechanisms of aerobic respiration. Accordingly, the genome of *G. bemidjiensis* Bemᵀ harbors genes that encode aerobic oxidation enzymes, including cytochrome c oxidase subunits I, II, III, and IV (encoded by *coxA*, *B*, *C*, and *D*, respectively; and NC_011146.1: Gbem_0043, 0046, 0044, and 0045, respectively)¹⁰, a cytochrome c oxidase synthesis (SCO) factor encoded by *sco* (Gbem_0042), and antioxidant enzymes, such as the iron/manganese-containing superoxide dismutase encoded by *sodA* (Gbem_2204).

Growth of anaerobically grown starter cultures of *G. bemidjiensis* Bemᵀ (approximately 50 mg dry cells) was not achieved following direct inoculation into media prepared in flasks under normal oxic conditions in our study (data not shown). Therefore, aerobic growth may require gradual acclimation of anaerobically grown cells (ambient conditions) under conditions similar to those of the present type 3 cultures.

Aerobic cultivation of *G. bemidjiensis* Bemᵀ did not generate qualitative changes in fatty acid and HC compositions, and the corresponding quantitative changes were minimal (Table 1). However, cells from type 3 cultures contained lower levels of 16:0 and 16:1(9c) than cells from type 1 and 2 cultures, whereas 14:0 and 14:1(7c) levels were higher in type 3 cultures than in type 1 and 2 cul-

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**Table 2** Parent and molecular fragment ions (m/z) of hopanes in fatty acid and hydrocarbon fractions from *G. bemidjiensis* Bemᵀ and *G. metallireducens* DSM 7210ᵀ.

<table>
<thead>
<tr>
<th>Hopene derivatives</th>
<th>Characteristic molecular ions (m/z)*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hop-17(21)-ene from Bemᵀ</td>
<td>+S +S +S +S - +W - +B - +W +W -</td>
<td>This study</td>
</tr>
<tr>
<td>Hop-17(21)-ene from DSM 7210ᵀ</td>
<td>+S +S +S +S - +W - +B - +W +W -</td>
<td>This study</td>
</tr>
<tr>
<td>Hop-17(21)-ene from the database⁶</td>
<td>+S +S +S +S - +W - +B - +W +W -</td>
<td>This study</td>
</tr>
<tr>
<td>Hop-21-ene from DSM 7210ᵀ</td>
<td>ND ND + ND - - + - - - ND + W -</td>
<td>Härtner et al.¹⁵</td>
</tr>
<tr>
<td>Hop-22(29)-ene from DSM 7210ᵀ</td>
<td>ND ND + ND - - + - - - ND + W -</td>
<td>Härtner et al.¹⁵</td>
</tr>
<tr>
<td>Bishomohopan-32-ol acetate from DSM 7210ᵀ</td>
<td>ND ND + ND - - + - - - ND - +</td>
<td>Härtner et al.¹⁵</td>
</tr>
</tbody>
</table>

* Ion intensities: +B, base ion (100% intensity); +S, strong (>50% of the base ion intensity); and +W, weak (<50% of the base ion intensity; +, present; -, absent

² Molecular (Parent) ion
³ Data retrieved from Saturn Software Workstation ver. 5.52
⁴ ND, no data available

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**Fig. 4** Partial electron impact gas chromatogram of the FAME plus HC fraction prepared from *G. bemidjiensis* Bemᵀ (a), and the mass fragment profiles of HC₁₀ (hop-17(21)-ene) (b) and HC₁₀ (hopanoid-like compound) (c). The arrows indicate a hopanoid-diagnostic mass fragment ion at m/z 191⁶,³³.


Effects of aerobiosis on Geobacter

4 CONCLUSION

The results of this study show that G. bemidjiensis Bem\textsuperscript{T} grew in aerobic conditions after anaerobic cultivation, and the fatty acid compositions were altered in type 3 culture compared with those in type 1 and 2 cultures. LC-PUFAs such as EPA and DHA were absent, whereas C31:9 was detectable. This finding indicated that the \textit{pfA} genes are involved in the synthesis of C31:9. The relative abundance of fatty acids with shorter chain lengths including 14:0 and 14:1(\textit{t}c), increased in type 3 culture. These results indicate that aerobiosis affects the FA composition and \textit{de novo} FA synthesis, and that \textit{G. bemidjiensis} Bem\textsuperscript{T} would therefore be useful for elucidating mechanisms for aerobiosis. Overall, the relationship between the fatty acid composition and aerobiosis in \textit{G. bemidjiensis} Bem\textsuperscript{T} should be further clarified in future studies.

5 Acknowledgements

This work was supported in part by the National Institute of Polar Research, Japan and by a Grant-in-Aid for Scientific Research ((C)no. 22570130) from the Ministry of Education, Science, Sports, and Culture of Japan. We appreciate the technical assistance provided by Mr. Hiroyuki Tada.

Supporting Information

This material is available free of charge via the Internet at http://dx.doi.org/10.5650/jos.ess.16122

References


