**Sedimenticola thiotaurini** sp. nov., a sulfur-oxidizing bacterium isolated from salt marsh sediments, and emended descriptions of the genus **Sedimenticola** and **Sedimenticola selenatireducens**

Beverly E. Flood, Daniel S. Jones and Jake V. Bailey

A marine facultative anaerobe, strain SIP-G1\(^T\), was isolated from salt marsh sediments, Falmouth, MA, USA. Phylogenetic analysis of its 16S rRNA gene sequence indicated that it belongs to an unclassified clade of **Gammaproteobacteria** that includes numerous sulfur-oxidizing bacteria that are endosymbionts of marine invertebrates endemic to sulfidic habitats. Strain SIP-G1\(^T\) is a member of the genus **Sedimenticola**, of which there is one previously described isolate, **Sedimenticola selenatireducens** AK4OH1\(^T\). **S. selenatireducens** AK4OH1\(^T\) was obtained for further characterization and comparison with strain SIP-G1\(^T\). The two strains were capable of coupling the oxidation of thiosulfate, tetrathionate, elemental sulfur and sulfide to autotrophic growth and they produced sulfur inclusions as metabolic intermediates. They showed varying degrees of O\(_2\) sensitivity, but when provided amino acids or peptides as a source of energy, they appeared more tolerant of O\(_2\) and exhibited concomitant production of elemental sulfur inclusions. The organic substrate preferences and limitations of these two organisms suggest that they possess an oxygen-sensitive carbon fixation pathway(s). Organic acids may be used to produce NADPH through the TCA cycle and are used in the formation of polyhydroxyalkanoates. Cell-wall-deficient morphotypes appeared when organic compounds (especially acetate) were present in excess and reduced sulfur was absent. Levels of DNA–DNA hybridization (\(~47\%\) and phenotypic characterization indicate that strain SIP-G1\(^T\) represents a separate species within the genus **Sedimenticola**, for which the name **Sedimenticola thiotaurini** sp. nov. is proposed. The type strain is SIP-G1\(^T\) (=ATCC BAA-2640\(^T\)=DSM 28581\(^T\)). The results also justify emended descriptions of the genus **Sedimenticola** and of **S. selenatireducens**.

The genus **Sedimenticola** and its single described species, **Sedimenticola selenatireducens**, were originally proposed to describe an obligate anaerobe isolated from estuarine sediments that was capable of coupling the oxidation of aromatic compounds to selenate respiration (Knight et al., 2002; Narasingarao & Häggblom, 2006). However, this genus falls within an unclassified clade of the **Gammaproteobacteria** that contains many uncultured (micro)-aerobic sulfur-oxidizing endosymbionts of marine invertebrate animals (Dubilier et al., 2008). Genomic and expression analyses of some of these endosymbionts indicate that they have metabolically complete genomes (Kuwahara et al., 2007; Markert et al., 2007, 2011; Nakagawa et al., 2014; Newton et al., 2007; Plazzi et al., 2013; Stewart et al., 2011; Woyke et al., 2006) and some strains appear to have a motile free-living stage (Gros et al., 2012; Sanders et al., 2013). The paucity of isolates in this clade limits our understanding of their physiologies. In this study, we report that **S. selenatireducens**, and a new isolate (SIP-G1\(^T\)), which we propose represents a novel species of the genus **Sedimenticola**, are sulfur-oxidizing autotrophs, as suggested by their phylogenetic relationship to sulfur-oxidizing symbionts.

After four successive streaks for isolation, strain SIP-G1\(^T\) was isolated on aerobic marine broth 2216 (MB2216; Difco) agar plates incubated at room temperature from long-term, low-diversity anaerobic enrichments inoculated

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**Abbreviations:** DAPI, 4',6'-diamidino-2-phenylindol; PHA, polyhydroxyalkanoate; polyP, polyphosphate; TMAO, trimethylamine N-oxide.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SIP-G1\(^T\) is JN882289.
Genomic DNA for 16S rRNA gene sequencing of strain SIP-G1T was isolated using Qiagen’s Genomic-tip 500 kit. Genomic DNA for 16S rRNA gene sequencing of strain SIP-G1T and screened for chimeras utilizing Bellerophon v.3 assembled and error checked utilizing MacVector v.10.6, 3.1 chemistry. The resulting DNA sequences were sequenced using a 96 capillary 3730xl sequencer (Applied Biosystems) that employed ABI BigDye Terminator version 97.6 % sequence similarity (1392/1426 bases) whereas the 16tRNA gene sequence of ‘Thiolapillus brandeum’ Hiromi 1 was 92 % similar (1256/1366 bases). A loose cut-off around ~97% similarity between 16S rRNA gene sequences has been commonly used to define novel species (Stackebrandt & Goebel, 1994; Yarza et al., 2008, 2010) but more recently a similarity of 98.65 % has been suggested (Kim et al., 2014). Given the level of sequence similarity between the two strains, and the paucity of information on the genus Sedimenticola, S. selenatireducens AK4OH1T was obtained in this study for further characterization and comparative analyses with strain SIP-G1T.

Routinely, the two strains were maintained on MB2216, but at 50 % of the recommended concentration for S. selenatireducens AK4OH1T to simulate estuarine-like salinity. A defined basal medium was used for studies of substrate utilization and optimal growth conditions. Basal salt solution (per litre) contained 0.112 g CaCl2, 0.5 g KCl, 1.46 g MgSO4, 30 g NaCl and 0.23 g NH4Cl2 and was buffered with 1 ml of a 500 mM KPO4 solution, pH 7.5, as well as 20 mM MOPS. The base medium also included 1 ml of a 1000 × vitamin solution and 1000 × B12 solution (0.1 %) and 10 ml of a 100 × trace metals solution. The vitamin solution contained 100 mg each of thiamine HCl, l-ascorbic acid, calcium D-pantothenate, folic acid, biotin, nicotinic acid, 4-aminobenzoic acid, pyridoxine HCl, thiotropic acid, NAD and inositol dissolved in 100 ml of a 10 mM KPO4 buffer (pH 7). The trace metal solution (pH 7.7) contained (per litre) 0.1 g FeCl2·2H2O, 0.03 g H3BO3, 0.1 g MnCl2, 0.1 g CoCl2·6H2O, 1.5 g nitrolitriacetid acid, 0.002 g NiCl2·6H2O, 0.144 g ZnSO4·7H2O, 0.036 g NaMoO4, 0.025 g sodium vanadate, 0.010 g Na2SeO3 and NaWO4·2H2O. Sodium bicarbonate buffer concentrations in the defined media depended on atmospheric conditions: anaerobic, 70 mM; hypoxic, 35 mM; and aerobic, 10 mM. The final pH was 7.9–8.0. All stock solutions and final media were filter-sterilized with a 0.22 µm filter. Anaerobic media and stock solutions were prepared in an anaerobic chamber (Coy) with an N2/H2 gas mix and the gas phase of the sealed culture vessels was 20 % CO2 and 80 % N2 (~25 p.s.i.). Hypoxic media were made under aerobic conditions and incubated on a shaker in a hypoxic chamber (Coy) with ~5 % atmospheric level of O2. During early hypoxic experiments, CO2 in the chamber was below atmospheric concentrations and the media contained 10 mM NaHCO3. However, experimentation demonstrated that an increased CO2 concentration improved growth potential and so subsequent hypoxic physiology studies were performed with ~5 % CO2 in the hypoxic chamber and 35 mM NaHCO3 in the media.

While strain SIP-G1T was able to grow bench-top on MB2216 agar plates, S. selenatireducens AK4OH1T was incapable of growth under standard atmospheric O2 concentrations. However, S. selenatireducens AK4OH1T grew well under hypoxic conditions on MB2216 plates. Microscopy indicated that both strains form elemental sulfur inclusions when grown on MB2216 and in some cases colonies appear white with S6, which dissolves upon exposure to

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acetone and ethanol (Nielsen et al., 2000). Commonly, colonies appeared with a translucent orange colour with a metallic sheen. The orange pigmentation appeared to be, at least in part, the result of an ethanol-soluble protein that can be extracted into the supernatant when cultures are placed in 50% ethanol. During early to mid-exponential growth in liquid MB2216 medium, cells of both strains appeared as motile rods with diameters of 0.5 mm and lengths of 1–2 mm. A single sulfur inclusion was visible in the cell, usually at one pole. Gram staining (Aldon) indicated that both strains are Gram-stain-negative. In late exponential phase, cells exhibited a swollen, elongate and pleomorphic appearance, and were non-motile. During late exponential phase, the cells also appeared to contain granules of storage compounds. To identify the composition of these granules, late exponential phase cells were ethanol fixed to a slide, stained with 1 mM Nile Blue A and incubated in the dark over a 37 °C water bath for 30 min before being observed under an FITC filter set (excitation 494 nm, emission 518 nm) on an Olympus BX-61 compound microscope. The inclusions, which had a greenish colour under phase contrast microscopy, fluoresced under FITC, indicating the inclusions are composed of polyhydroxyalkanoates (PHAs). As PHA accumulation is commonly associated with polyphosphate (polyP) accumulation in wastewater models (Hauduc et al., 2013), fixed cells were stained throughout the growth curve for polyP with 4',6'-diamidino-2-phenylindol (DAPI) at 5 μg ml⁻¹ and examined under a broad pass DAPI filter set (excitation 345 nm, emission 455 nm), which revealed polyP accumulation occurred prior to PHA formation in the batch cultures (Streichan et al., 1990).

Optimal growth conditions were determined early in this study. To determine optimal salinity conditions both strains were grown under hypoxic conditions with both 5 mM...
sodium pyruvate and 1 mM NaS₂O₃ as electron donors in the basal medium buffered with 10 mM MOPS and 10 mM NaHCO₃. The NaCl concentrations tested were 0.1, 1.1, 2.3, 3.0, 3.5, 4.5, 5.5, 6.5 and 7.5 %. The optimal salinity for *S. selenatireducens* AK4OH¹⁷ was 2.3 % NaCl as previously reported, and between 3.0 and 3.5 % for strain SIP-G¹⁷. Neither strain grew with 0.1 % NaCl. The optimal temperatures for growth were assessed under anaerobic conditions with the addition of 10 mM NaNO₃ as the terminal electron acceptor and both 5 mM sodium pyruvate and 1 mM NaS₂O₃ as electron donors. The incubation temperatures were 12 °C, room temperature (~25 °C), and 30, 35, 37 and 45 °C. The optimal temperature for strain SIP-G¹⁷ was 35 °C, while *S. selenatireducens* AK4OH¹⁷ grew best at room temperature. Growth rates for each strain were determined at room temperature under anaerobic conditions with 5 mM NaS₂O₃, 5 mM sodium pyruvate and 10 mM NaNO₃ with 3.5 % NaCl for strain SIP-G¹⁷ and 2.3 % NaCl for *S. selenatireducens* AK4OH¹⁷. The generation time of strain SIP-G¹⁷ was 7 h and the generation time of *S. selenatireducens* AK4OH¹⁷ was 6 h.

Numerous attempts to grow *S. selenatireducens* AK4OH¹⁷ and strain SIP-G¹⁷ aerobically failed unless grown on MB2216 (*S. selenatireducens* AK4OH¹⁷ grew very poorly in unshaken broth), or on a defined medium as described above, but with either casamino acids or cysteine as an energy source. Both strains produced S⁰ inclusions when grown on cysteine. The formation of S⁰ inclusions when grown on peptone and cysteine suggests that these strains are sulfur-oxidizing bacteria. As many sulfur-oxidizing bacteria are autotrophic, sometimes with oxygen-sensitive carbon fixation pathways, we explored this metabolic potential. We also assessed the potential capacity for these oxidase-positive (Sigma test kit) strains to detoxify H₂O₂ produced by oxidase activity. Strain SIP-G¹⁷ and *S. selenatireducens* AK4OH¹⁷ were grown under hypoxic conditions and were then subjected to 3 % (v/v) H₂O₂. Bubble production, i.e. catalase activity, was not observed for *S. selenatireducens* AK4OH¹⁷ but was observed for strain SIP-G¹⁷.

Batch culturing confirmed that both strains oxidize S₂O₂³⁻, forming S⁰ inclusions (Fig. 2a), under hypoxic and denitrifying conditions with inorganic carbon as the sole source of carbon. However, one notable difference was that when grown in a hypoxic broth medium, strain SIP-G¹⁷ grew readily whereas *S. selenatireducens* AK4OH¹⁷ grew more slowly, suggesting that the latter is more O₂-sensitive. Strain SIP-G¹⁷ often formed a pellicle at the surface under hypoxic conditions. In contrast, *S. selenatireducens* AK4OH¹⁷ was typically found at the bottom of the culture vessel, indicating that the optimal O₂ concentration for this strain is below 5% atmospheric level. The capacity to form sulfuric acid as an end product of thiosulfate oxidation was tested on agar plates (buffered with 10 mM Tris/HCl, pH 8.0) containing 0.005% phenol red as a pH indicator and 5 mM thiosulfate as the sole source of energy. The

**Fig. 2.** (a) Elemental sulfur formation by *S. selenatireducens* AK4OH¹⁷ during S₂O₂³⁻ oxidation. (b) PHA accumulation in long rods of *S. selenatireducens* AK4OH¹⁷ when grown on acetate. Arrows indicate round cell-wall-deficient morphotypes. Aberrant cell-wall-deficient morphotype upper left. (c) Elongation of cells of strain SIP-G¹⁷; arrows show round cell-wall-deficient morphotypes. Bar, 10 µm; applies to all three images.
plates were incubated in the hypoxic chamber. Colonies were white with encrusting sulfur, and several days after colony formation the colour of the agar around the colonies changed from pink to yellow, indicating acid production.

The oxidation of 2 mM H$_2$S, 5 mM tetrathionate, 1% (w/v) sulfur, 1 mM sulfite and 5 mM cysteine was examined under anaerobic conditions with 10 mM nitrate serving as the electron acceptor. Both strains oxidized H$_2$S, tetrathionate and elemental sulfur and formed sulfur inclusions. *S. selenatireducens* grew well on sulfite whereas strain SIP-G1 did not grow on sulfite. Neither strain grew on cysteine even though it served as an electron donor when O$_2$ was an electron acceptor.

Electron acceptors for anaerobic respiration were determined with acetate as the electron donor. Both strains demonstrated positive growth with nitrite, nitrate and sulfate and strain SIP-G1 also respired trimethylamine N-oxide (TMAO) and DMSO. Neither strain respired chlorate, perchlorate or bromate when provided acetate as the sole electron acceptor.

Energy sources, chlorate and perchlorate served as electron acceptor of energy, yet when peptone was used as the sole source, perchlorate or bromate when provided acetate as the sole electron acceptor. Both strains oxidized H$_2$S, tetrathionate and elemental sulfur and formed sulfur inclusions. *S. selenatireducens* grew well on sulfite whereas strain SIP-G1 did not grow on sulfite. Neither strain grew on cysteine even though it served as an electron donor when O$_2$ was an electron acceptor.

Electron acceptors for anaerobic respiration were determined with acetate as the electron donor. Both strains demonstrated positive growth with nitrite, nitrate and sulfate and strain SIP-G1 also respired trimethylamine N-oxide (TMAO) and DMSO. Neither strain respired chlorate, perchlorate or bromate when provided acetate as the sole source of energy, yet when peptone was used as the sole energy source, chlorate and perchlorate served as electron acceptors for both strains while bromate served as an electron acceptor only for strain SIP-G1. Respiration using these three electron acceptors yields O$_2$ as a metabolic end product (Coates & Achenbach, 2004), the production of which may inhibit carbon fixation. Neither strain respired sulfite or sulfate. When grown anaerobically, both organisms appeared non-motile under the microscope. However, after prolonged exposure to O$_2$ (20–30 min), exponentially growing cells often became motile. These findings, along with observations of the cultures in the hypoxic chamber, suggest that both strains possess chemotactic responses to O$_2$.

Biolog’s phenotype characterization microarray plate PM-1 was utilized to elucidate carbon source utilization. Colonies grown at room temperature on MB2216 agar plates under hypoxic conditions were suspended in the basal medium with optimal salinity conditions, 35 mM NaHCO$_3$, 5 mM NH$_4$NO$_3$, 0.1 g casamino acids l$^{-1}$ and Biolog’s dye H. Triplicate plates were examined daily for more than 5 days. A positive metabolic response was indicated by a change in colour from clear to pink and if robustly utilized to a deep purple or magenta resulting from the reduction of tetrazolium by NADH. The organic acids formate, acetate, pyruvate, L-lactate, D-malate, D-malate, propionate, fumarate and succinate stimulated NADH production by both strains, but citrate did not. Both strains responded positively to methylated succinate and pyruvate, bromated succinate and the aromatic p-hydroxyphenylacetic acid.

They also responded positively to 2-ketoglutaric acid, but much less so to 2-ketobutyric acid or 2-hydroxybutyric acid. Both strains demonstrated a rapid response to the C5 sugars ribose, xylose, lyxose and arabinose, but the colour change halted within 2 days and remained a faint pink. Similarly rapid but faint colour changes were observed for both strains in the presence of d-galacturonic acid γ-lactone and for *S. selenatireducens* AK4OH1T in the presence of 2-hydroxyglutaric acid γ-lactone. Strain SIP-G1T demonstrated a significant delay in the catabolism of Tween 20 but yielded a strong colour change (> 5 days).

The two strains were markedly different in amino acid preferences with the exception that both strains responded favourably to L-alanine, and less so to L-serine and L-glutamic acid. Additionally, colour changes were delayed in both strains on amino acids relative to the rapid colour change observed in the presence of C5 sugars and organic acids. Tyramine, and to a small extent phenylethylamine, produced positive reactions with *S. selenatireducens* AK4OH1T. D-Alanine, L-asparagine, L-glutamine and the peptide glycyl L-aspartic acid were favourable for strain SIP-G1T. The following PM-1 substrates were not hydrolysed by either strain: D-glucuronic acid, D-glucaric acid, thymidine, uridine, 2-deoxyadenosine, adenosine, inosine, N-acetyl-D-glucosamine, D-glucosaminic acid, N-acetyl-β-D-mannosamine, p-hydroxyphenylacetic acid, L-galactonic acid γ-lactone, L-saccharic acid, trehalose, maltose, melibiose, lactulose, sucrose, cellobiose, D-galactose, D-mannose, D-fructose, z-D-glucose, z-lactose, psicose, dulcitol, D-sorbitol, glycerol, D-mannititol, adonitol, myo-inositol, L-fucose, D-rhamnose, glycerol 3-phosphate, D-glucose 6-phosphate, D-glucose 1-phosphate, D-fructose 6-phosphate, methyl z-D-galactopyranoside, Tween 40 and 80, methyl β-D-galactopyranoside, 1,2-propanediol, mucic acid, myo-tartaric acid, glyoxylic acid, glycolic acid, tricarboxylic acid, acetoacetic acid, D-galacturonic acid, maltotriose, glucuronamide, ethanolamine, L-proline, L-threonine, L-asparagine, D-aspartic acid, L-aspartic acid, L-glutamine, L-threonine, L-α-aminol glycine, glycerol L-proline, glycol L-glutamic acid and m-hydroxyphenylacetic acid.

Batch culturing to determine substrate utilization and niche preferences proved to be challenging. Due to intracellular storage of polyP and PHAs and the production of S$_0$, transferring a culture to a new medium/condition often yielded an increased optical density even though the medium was unsuitable for growth. Microscopy revealed that in some cases a change in optical density was driven by the accumulation of stored substrates resulting in multiple cell divisions. Negative controls (i.e. base media minus a test substrate) were essential to interpreting growth preferences. For this reason, colour changes rather than absorbance were reported for the Biolog assay. Organic acid preferences (malate, lactate, pyruvate and succinate, but not citrate) were confirmed by batch culturing for both strains. Attempts to grow both strains with ribose or arabinose as a sole source of carbon failed; however, addition of casamino acids to ribose or arabinose did stimulate growth above that observed with casamino acids alone.

The accumulation of PHAs was particularly troublesome when both strains were grown repeatedly on organic acids as the sole electron donor because the cells became elongated, highly pleomorphic and swollen with PHAs (Fig. 2b). Under these conditions, the cells were highly fragile and lysed easily during microscopic observation.
Successive batch culturing with acetate as the sole electron donor under denitrifying conditions led to the loss of viability of both strains. However, when both strains were grown in the presence of both an organic acid and thiosulfate as energy sources, the aberrant growth and excessive PHA accumulation either did not occur or were reduced. Instead, there was a tendency for both cultures to form adherent biofilms under hypoxic conditions. Recently it was suggested that all sulfur-oxidizing \textit{Gammaproteobacteria} use the TCA cycle as a mechanism to assimilate short chain fatty acids to reduce the energetic costs of carbon fixation and to produce NADPH (in preference to the pentose phosphate pathway) as demonstrated in ‘Thiolla\-pillus brandeum’ Hiromi 1 (Nunoura \textit{et al.}, 2014). Another potential benefit of assimilating TCA cycle intermediates appears to be the production of PHAs (Zhou \textit{et al.}, 2009, 2010), which would be particularly beneficial to strains posing the potential benefit of assimilating TCA cycle intermediates. However, when both strains were grown in the presence of both an organic acid and thiosulfate as energy sources, the aberrant growth and excessive PHA accumulation either did not occur or were reduced. Instead, there was a tendency for both cultures to form adherent biofilms under hypoxic conditions. Recently it was suggested that all sulfur-oxidizing \textit{Gammaproteobacteria} use the TCA cycle as a mechanism to assimilate short chain fatty acids to reduce the energetic costs of carbon fixation and to produce NADPH (in preference to the pentose phosphate pathway) as demonstrated in ‘Thiolla\-pillus brandeum’ Hiromi 1 (Nunoura \textit{et al.}, 2014). Another potential benefit of assimilating TCA cycle intermediates appears to be the production of PHAs (Zhou \textit{et al.}, 2009, 2010), which would be particularly beneficial to strains possessing the potential benefit of assimilating TCA cycle intermediates.

Table 1. Distinguishing characteristics between strain SIP-G1T and \textit{S. selenatireducens} AK4OH1T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SIP-G1T</th>
<th>\textit{S. selenatireducens} AK4OH1T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal temperature; salinity</td>
<td>35 °C; 3.5% NaCl</td>
<td>25 °C; 2.3% NaCl</td>
</tr>
<tr>
<td>Inorganic electron donors</td>
<td>H₂S, S₂O₂⁻²⁻, tetrahionate, S⁰</td>
<td>H₂S, S₂O₂⁻²⁻, tetrahionate, S⁰, SO₃²⁻</td>
</tr>
<tr>
<td>Organic electron donors under aerobic conditions</td>
<td>Peptone, casamino acids, cysteine</td>
<td>Peptone, casamino acids, * cysteine*</td>
</tr>
<tr>
<td>Electron acceptors</td>
<td>O₂, NO₃⁻⁻, NO₂⁻⁻, ClO₄⁻⁻,† ClO₃⁻⁻,† TMAO, DMSO, SeO₄⁻⁻,† BrO₃⁻⁻,†</td>
<td>O₂, NO₃⁻⁻, NO₂⁻⁻, ClO₄⁻⁻,† ClO₃⁻⁻,† SeO₄⁻⁻</td>
</tr>
<tr>
<td>Polar lipids</td>
<td>Phosphatidylethanolamine</td>
<td>Phosphatidylethanolamine</td>
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<tr>
<td></td>
<td>Phosphatidyldiglycerol</td>
<td>Phosphatidyldiglycerol</td>
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<tr>
<td></td>
<td>Diphosphatidylylglycerol</td>
<td>Two U/I aminophospholipids</td>
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<tr>
<td></td>
<td>Three U/I aminophospholipids</td>
<td>Phospholipids</td>
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<tr>
<td></td>
<td>Two U/I phospholipids</td>
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<tr>
<td></td>
<td>Three other U/I lipids</td>
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<tr>
<td>Fatty acids</td>
<td>C₁₀:₀ 3- OH (1.4 %)</td>
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<td></td>
<td>U/I (ECL 11.800) (2.7 %)</td>
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<tr>
<td></td>
<td>C₁₂:₀ 3- OH (1.6 %)</td>
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<tr>
<td></td>
<td>C₁₆:₁, ε₁₇ (41.5 %)</td>
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<tr>
<td></td>
<td>C₁₆:₀ (28.1 %)</td>
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<tr>
<td></td>
<td>C₁₈:₁, ε₁₇ (21.2 %)</td>
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</tr>
<tr>
<td>Quinone(s)</td>
<td>Q7 (14 %), Q8 (86 %), MK-8</td>
<td>Q7</td>
</tr>
</tbody>
</table>

*Weak response.
†Growth requires a substrate specific for aerobic growth.

Both isolates were harvested under denitrifying conditions, with acetate as a source of energy. They were then characterized on DNA–DNA hybridization, peptidoglycan content, quinone profiling and polar lipid profiling (performed by the Identification Service of the DSMZ, Braunschweig, Germany). In addition, a fatty acid profile of strain SIP-G1T was also provided by the DSMZ (Narasingarao & Haggblom, 2006). Cellular profiles of quinones, polar lipids (TLC profiles are shown in Fig. S1, available in the online Supplementary Material) and fatty acids are presented in Table 1. For the peptidoglycan analyses, the amino acids of the total hydrolysate (100 °C, 4 M HCl, 16 h) of the whole cells were derivatized to N-heptafluorobutyric amino acid isobutyl esters and analysed by GC/MS (Schumann, 2011). Subsequent polymeric material was also treated by hydrolysis and derivatization for GC/MS analysis. Both attempts failed to detect traces of dianimopimelic acid or muramic acid, although a large spectrum of proteinogenic amino acids was detected. Thus, these strains may possess little, if any, peptidoglycan when grown with acetate as a carbon source. As flagellated propelled motility has been observed, it seems probable that the loss of peptidoglycan was responsible for the pleomorph observed when provided with organic acids, especially acetate, as the electron donor. Pleomorphic or cell-wall-deficient forms are shown in Fig. 2(b,c). Cell-wall-deficient morphotypes were also observed under a variety of culture conditions but often confined to a smaller fraction of the population. Strains capable of producing cell-wall-deficient forms, although poorly understood, are commonly pathogens or symbionts (Allan \textit{et al.}, 2009; Briers \textit{et al.}, 2012). A recent study of a model Gram-positive cell-wall-deficient strain found that membrane fluidity, in particular branched fatty acids, was essential for cell division in cell-wall-deficient morphotypes.
Emended description of Sedimenticola

This description emends that of Narasingarao & Häggblom (2006). Cells are motile rods that elongate and become morphologically variable, and can include cell-wall-deficient morphotypes when grown with organic substrates. At least part of the morphological variability appears to be due to polyP and PHA accumulation. Cells are Gram-stain-negative and oxidase-positive. Respiration is supported by O2, chloride and perchlorate when peptone serves as a carbon source. Chemolithoautotrophic growth occurs with thiosulfate under hypoxic and anaerobic conditions, but not under atmospheric air. Variable chemotaxis responses to O2 depend on electron donor and O2 concentration. Chemolithoautotrophic growth also occurs with H2S, tetraethionate and elemental sulfur. Elemental sulfur inclusions are formed as an intermediate during sulfur oxidation. Colonies grown on MB2216 agar appear translucent orange with a metallic sheen, and deposits of elemental sulfur are sometimes present.

Emended description of Sedimenticola selenatireducens

Cells are catalase-negative. Sulfite serves as an electron donor. Substrate preferences connected to the TCA cycle include formate, D-malate, L-malate, propionate, fumarate and succinate. Other substrates include cysteine, methyl succinate, methyl pyruvate, bromosuccinate, p-hydroxyphenylacetic acid, thiotaurine; N.L. neut. n. thiotaurinum thiotaurine; N.L. gen. n. thiotaurini pertaining to thiotaurine).

Cells are catalase-positive. Sulfite does not serve as an electron donor. Terminal electron acceptors include TMAO, DMSO and bromate (poor growth) if peptone is provided as a carbon source. Substrate preferences connected to the TCA cycle include formate, D-malate, L-malate, propionate, fumarate and succinate. Other substrates include cysteine, methyl succinate, methyl pyruvate, bromosuccinate, p-hydroxyphenylacetic acid, Tween 20, 2-ketoglutaric acid, D-alanine, L-asparagine, L-glutamine, glycerol, L-aspatic acid and L-alanine. Weak responses occur with L-serine, L-glutamic acid, 2-ketobutyric acid, 2-hydroxybutyric acid, ribose, xylose, lyxose, arabinose and D-galactonic acid γ-lactone. Other substrates in Biolog’s phenotype characterization microarray plate PM-1 are not utilized. Major quinones are Q7, Q8 and MK-8. The major fatty acids are C16:1ω7c, C16:0, C18:1ω7c and an unknown fatty acid (ECL 11.800); C10:0 3-OH and C12:0 3-OH are minor constituents. Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol, while two unidentified phospholipids, three unidentified aminophospholipids and three other unidentified lipids are minor components of the lipid profile.

The type strain is SIP-G1T (=ATCC BAA-2640T = DSM 28581T), isolated from salt marsh sediments from the Sippiwissett Salt Marsh, Falmouth, Massachusetts, USA.

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References


Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the


