Sunxiuqinia faeciviva sp. nov., a facultatively anaerobic organoheterotroph of the Bacteroidetes isolated from deep subseafloor sediment

Ken Takai,1 Mariko Abe,1 Masayuki Miyazaki,1 Osamu Koide,2 Takuro Nunoura,1 Hiroyuki Imachi,1 Fumio Inagaki3 and Tohru Kobayashi2

1Subsurface Geobiology Advanced Research (SUGAR) Project, Institute of Biogeosciences, Japan Agency for Marine-Earth Science & Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka 237-0061, Japan
2Soft Matter and Extremophiles Research Team, Institute of Biogeosciences, Japan Agency for Marine-Earth Science & Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka 237-0061, Japan
3Geomicrobiology Group, Kochi Institute of Core Sample Research, Japan Agency for Marine-Earth Science & Technology (JAMSTEC), Monobe B200, Nankoku, Kochi 783-8502, Japan

A facultatively anaerobic organoheterotroph, designated JAM-BA0302T, was isolated from a deep subseafloor sediment at a depth of 247.1 m below the seafloor off the Shimokita Peninsula of Japan in the north-western Pacific Ocean (Site C9001, water depth 1180 m). Cells of strain JAM-BA0302T showed gliding motility and were thin, long rods with peritrichous fimbria-like structures. Growth occurred at 4–37 °C (optimum 30 °C; doubling time 8 h), at pH 5.4–8.3 (optimum pH 7.5) and with 5–60 g NaCl l⁻¹ (optimum 20–25 g l⁻¹). The isolate utilized proteinaceous substrates such as yeast extract, tryptone, casein and Casamino acids with O₂ respiration or fermentation. Strain JAM-BA0302T was a piezotolerant bacterium that could grow at pressures as high as 25 MPa under aerobic conditions and 10 MPa under anaerobic conditions. The G+C content of the genomic DNA was 43.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain JAM-BA0302T was most closely related to yet-undescribed strains recently isolated from various marine sedimentary environments (>99.6 % 16S rRNA gene sequence similarity) and was moderately related to Sunxiuqinia elliptica DQHS-4T, isolated from a sea cucumber farm sediment (95.5 % 16S rRNA gene sequence similarity) within the Bacteroidetes. The phylogenetic analysis suggested that the isolate should belong to the genus Sunxiuqinia. However, low DNA–DNA relatedness (<11 %) and many physiological and molecular properties differentiated the isolate from those previously described. We propose here a novel species of the genus Sunxiuqinia, with the name Sunxiuqinia faeciviva sp. nov. The type strain is JAM-BA0302T (=JCM 15547T =NCIMB 14481T).

To date, evidence has been accumulating that marine subsurface sedimentary environments harbour an enormous microbial biomass, comprising a significant fraction of the total living biomass on Earth (D’Hondt et al., 2009; Lipp et al., 2008; Parkes et al., 2000; Whitman et al., 1998). Many studies have clarified the widespread occurrence of great functional and phylogenetic diversity of prokaryotes in global deep subseafloor sedimentary environments, most of which are as-yet-uncultivated members from other microbial habitats (as reviewed by Fry et al., 2008; Inagaki & Nakagawa, 2008; Inagaki, 2010; Jørgensen & Boetius, 2007; Parkes et al., 2000; Teske, 2006). Despite the great phylogenetic and functional diversity being evident, very few micro-organisms have been described from deep or even shallow subseafloor sediments (Bale et al., 1997; Kendall et al., 2006; Lee et al., 2005; Mikucki et al., 2003; Takai et al., 2005; Toffin et al., 2004).

Although the physiological characteristics of most subseafloor sedimentary micro-organisms remain unknown, previous biogeochemical and molecular ecological studies have demonstrated that the microbial biomass in subseafloor sediments is highly dependent on organic matter produced by photosynthetic organisms in the overlying
seawater, suggesting that subseaﬂoor microbial organoheterotrophy plays an important ecological role in Earth’s carbon cycle (D’Hondt et al., 2002, 2009; Biddle et al., 2006; Inagaki et al., 2006; Lipp et al., 2008). Indeed, cultivation-dependent characterization of deep subseaﬂoor sediments has yielded a diversity of aerobic and anaerobic organoheterotrophic bacteria (Batzke et al., 2007; D’Hondt et al., 2004; Kobayashi et al., 2008; Parkes et al., 2009). Most of these deep subseaﬂoor organoheterotrophs are members of the Firmicutes, Actinobacteria, Bacteroidetes, Alphaproteobacteria and Gammaproteobacteria and are closely related to the previous described species from the marine and terrestrial environments on the Earth’s surface biosphere (Batzke et al., 2007; D’Hondt et al., 2004; Kobayashi et al., 2008; Parkes et al., 2009).

Of those isolates previously reported from subseaﬂoor sediments, however, some exhibit distant phylogenetic relationships with previously described species, and most are afﬁliated with the phylum Bacteroidetes (Batzke et al., 2007; Kobayashi et al., 2008; Parkes et al., 2009). In particular, a certain phylogenetic group of organoheterotrophic Bacteroidetes strains have been isolated from geographically distinct subseaﬂoor sedimentary habitats in northeast Japan (Kobayashi et al., 2008) and the Indian Continental Shelf (Parkes et al., 2009), and even from mineral deposits associated with the deep crustal fluids in the Juan de Fuca Ridge ﬂank (Nakagawa et al., 2006). Within the Bacteroidetes, only Meniscus glaucopsis (Irgens, 1977) and Prolixibacter bellariivorans (Holmes et al., 2007), both facultatively anaerobic mesophilic organoheterotrophs, are related to the phylotypes of subseaﬂoor isolates. Recently however, several strains representing a novel species in a new genus of the Bacteroidetes, Sunxiuqinia elliptica, were isolated from sea cucumber farm sediment on the east coast of China and this strictly aerobic organoheterotroph is most closely related to the subseaﬂoor Bacteroidetes isolates (Qu et al., 2011). This study characterizes strain JAM-BA0302T, which represents the subseaﬂoor Bacteroidetes phylgroup and was isolated from a core section of a deep sediment (Kobayashi et al., 2008).

Strain JAM-BA0302T was isolated from a methan hydrate-bearing layer of sediment at a depth of 247.1 m below the seafloor in the deep sea (water depth 1180 m) off the Shimokita Peninsula (Kobayashi et al., 2008). The sediment sample was recovered from Site C9001 (41°10.63800’ N 142°12.081’ E) during the DV Chikyu shakedown expedition CK06-06 in 2006 (Aoike, 2007). The core sediment was obtained with a hydraulic piston coring system. Immediately after core recovery, 50 cm3 of seawater, suggesting that subseaﬂoor microbial organoheterotrophy plays an important ecological role in Earth’s carbon cycle (D’Hondt et al., 2002, 2009; Biddle et al., 2006; Inagaki et al., 2006; Lipp et al., 2008). Indeed, cultivation-dependent characterization of deep subseaﬂoor sediments has yielded a diversity of aerobic and anaerobic organoheterotrophic bacteria (Batzke et al., 2007; D’Hondt et al., 2004; Kobayashi et al., 2008; Parkes et al., 2009). Most of these deep subseaﬂoor organoheterotrophs are members of the Firmicutes, Actinobacteria, Bacteroidetes, Alphaproteobacteria and Gammaproteobacteria and are closely related to the previous described species from the marine and terrestrial environments on the Earth’s surface biosphere (Batzke et al., 2007; D’Hondt et al., 2004; Kobayashi et al., 2008; Parkes et al., 2009).

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After puriﬁcation, strain JAM-BA0302T was routinely cultivated in MJYTG medium (pH 7.5) consisting of 2 g yeast extract, 2 g tryptone and 0.5 g glucose per litre of MB synthetic seawater (Takai et al., 1999) or in marine broth 2216 (MB; pH 7.5).

Cells were routinely observed under a phase-contrast BX51 microscope with a DP71 digital camera system (Olympus). Transmission electron microscopy of negatively stained cells and thin sections of cells was carried out as described by Zillig et al. (1990). Cells of strain JAM-BA0302T were aerobically grown in MB at 30 °C to the mid-exponential and stationary phases of growth for microscopic observation with a JEOL JEM-1210 electron microscope. The cells were thin, long rods (0.5–0.7 μm in width and 4–28 μm in length) with peritrichous fimbriae-like structures and no flagella (Fig. 1a). In the late exponential to stationary phases of growth, spherical cells (0.7–0.9 μm wide) were frequently observed (Fig. 1b). Motility was not evident in liquid culture but gliding motility was observed on solid medium. No spore formation was observed under any of the growth conditions examined. Thin-section of electron micrographs showed a typical Gram-negative cell envelope structure (Fig. 1c). The colonies showed red pigmentation.

The isolate was routinely cultivated in MJYTG medium (pH 7.5) under air (atmospheric pressure) or under a gas phase of 100% N2 (2 atm) for aerobic and anaerobic growth, respectively. Growth was measured by direct cell counting after staining with 4′,6-diamidino-2-phenylindole (Porter & Feig, 1980) using a phase-contrast BX51 microscope unless otherwise noted. Cultures were usually grown in 15-ml glass tubes without shaking in a temperature-controlled dry incubator. The effects of temperature, pH and NaCl concentration on growth were determined using a Bio-Photorecorder (type TVS126MA; ADVANTEC) under aerobic conditions using MJYTG medium in 25-ml glass tubes with shaking, with the pH adjusted with 0.1 M HCl or NaOH at room temperature. Strain JAM-BA0302T grew at 4–37 °C (optimum 30 °C). The generation time at 30 °C and pH 7.5 was about 8 h (Fig. S1a, available in IJSEM Online). Strain JAM-BA0302T grew at pH 5.4–8.3 (optimum pH 7.5) (Fig. S1b) and with 5–60 g NaCl l−1 (optimum 20–25 g l−1) at 30 °C and pH 7.5 (Fig. S1c).

Strain JAM-BA0302T was isolated under aerobic cultivation conditions, while an anaerobic strain with an identical 16S rRNA gene sequence of strain JAM-BA0302T was obtained when the same sample was cultivated at 15 °C with MJYTG medium (pH 7.5) under a 100% N2 atmosphere (data not shown). The anaerobic growth capability of strain JAM-BA0302T was examined in the absence or presence of potential electron acceptors. Anaerobic fermentative growth was tested using nitrate-removed MJYTG medium under a gas mixture of 100% N2
respiration was tested using MJYTG medium supple-mented with each of the potential electron acceptors, such as 1 % (w/v) elemental sulfur, 20 mM thiosulfate, 10 mM nitrate, 10 mM fumarate and 30 mM ferric iron hydroxide. In this case, specific respiration substrates (20 mM acetate, lactate, pyruvate and succinate) were used instead of yeast extract, tryptone and glucose. Strain JAM-BA0302<sup>T</sup> fermentatively grew under anaerobic conditions but did not grow with any of the electron acceptors using organic acids as the energy source (Table 1). In addition, of the electron acceptors tested, none provided higher growth rate or yield than observed for fermentative growth in normal complex MJYTG medium. The results indicated that strain JAM-BA0302<sup>T</sup> was a facultative anaerobe, with O<sub>2</sub> respiration and fermentation of complex organic compounds.

Organoheterotrophic substrates for O<sub>2</sub> respiration and fermentation of strain JAM-BA0302<sup>T</sup> were tested using MJYTG medium under air (atmospheric pressure) and a gas phase of 100 % N<sub>2</sub> (2 atm), respectively, in which yeast extract, tryptone and glucose were replaced with each of the following organic compounds: (w/v) 0.2 % yeast extract, 0.2 % tryptone, 0.2 % casein, 0.2 % Casamino acids, 0.2 % starch, 0.1 % chitin, 0.1 % cyclodextrin, 0.05 % glucose, 0.05 % maltose, 0.1 % sucrose, 0.1 % galactose, 0.1 % lactose, 0.1 % fructose and 0.1 % xylose, (v/v) 0.2 % ethanol and 0.2 % glycerol, 20 mM pyruvate, 20 mM acetate, 20 mM formate, 20 mM tartrate, 20 mM succinate, 20 mM lactate and 10 mM each of the 20 amino acids. Under aerobic conditions, strain JAM-BA0302<sup>T</sup> grew with yeast extract, tryptone, casein, Casamino acids and glutamine while anaerobic fermentative growth was supported only with yeast extract. None of the other organic compounds supported both the aerobic and anaerobic growth of the isolate. Thus, strain JAM-BA0302<sup>T</sup> was an organoheterotroph utilizing complex proteinaceous compounds and glutamine for O<sub>2</sub> respiration and yeast extract as an anaerobic fermentation substrate.

The effect of hydrostatic pressure on growth was examined with MJYTG medium (pH 7.5) at 30 °C under aerobic and anaerobic conditions using a high-pressure cultivation technique described by Takai et al. (2008). The optimal soluble O<sub>2</sub> concentration (0.8 mM) in the medium was empirically determined. Thus, a high-pressure cultivation syringe contained 4 ml MJYTG medium (pH 7.5) with cell inoculum and 0.4 ml air (for aerobic growth) or 0.4 ml N<sub>2</sub> (for anaerobic growth). Under anaerobic conditions, strain JAM-BA0302<sup>T</sup> grew at 0.1, 5 and 10 MPa (highest growth rate at 5 MPa), and under aerobic conditions, at up to 25 MPa (highest growth rate at 0.1 MPa, but about 50 % growth rate even at 25 MPa). The results indicate that strain JAM-BA0302<sup>T</sup> is a piezotolerant bacterium that can survive and grow under pressure of its isolation habitat (14–15 MPa).

Antibiotic susceptibility of strain JAM-BA0302<sup>T</sup> was tested using MJYTG medium containing various antibiotics.
under aerobic and anaerobic conditions. Strain JAM-BA0302<sup>T</sup> was sensitive to (\(\mu g \text{ ml}^{-1}\)) chloramphenicol (50), kanamycin (100) and ampicillin (50) and resistant to streptomycin (100) under aerobic conditions and resistant to streptomycin (100) and kanamycin (100) under anaerobic conditions.

Oxidase activity was determined under aerobic conditions by spreading cells grown in MB at 30 °C to the late-exponential growth phase on oxidase test paper (Nissui Pharmaceutical). Catalase activity was determined using a cell pellet and observing bubble production from 3 % (v/v) \(H_2O_2\). Strain JAM-BA0302<sup>T</sup> was catalase-positive but oxidase-negative.

The chemotaxonomic properties of the isolate were analysed using cells grown aerobically in MB at 30 °C to the late-exponential growth phase. The type strains of closely related species, \(P.\ bellariivorans\) JCM 13498<sup>T</sup> (Holmes et al., 2007 and this study); 3, \(P.\ bellariivorans\) F2<sup>T</sup> (Holmes et al., 2007 and this study). E, Elliptical; FA, facultatively anaerobic; R, red; SA, strictly aerobic; SCR, straight to curved rod; W, white; WOR, white to orange-red; +, positive; −, negative; ND, no data available.

Table 1. Comparison of characteristics of strain JAM-BA0302<sup>T</sup> and its closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td><strong>Cell characteristics</strong></td>
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<tr>
<td>Shape</td>
<td>SCR</td>
<td>E</td>
<td>SCR</td>
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<tr>
<td>Dimensions ((\mu m))</td>
<td>0.5–0.7 × 4–28</td>
<td>0.4 × 0.8–0.9</td>
<td>0.33 × 10.5–12.5</td>
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<tr>
<td>Motility</td>
<td>Gliding</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Colour</td>
<td>R</td>
<td>WOR</td>
<td>W</td>
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<tr>
<td>Fimbriae-like structures</td>
<td>+</td>
<td>ND</td>
<td>–</td>
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<tr>
<td>Growth response to (O_2)</td>
<td>FA</td>
<td>SA</td>
<td>FA</td>
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<tr>
<td>Temperature for growth (°C)</td>
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<td></td>
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<tr>
<td>Range</td>
<td>4–37</td>
<td>15–42</td>
<td>4–42</td>
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<tr>
<td>Optimum</td>
<td>30</td>
<td>30</td>
<td>22</td>
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<tr>
<td>pH for growth</td>
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<tr>
<td>Range</td>
<td>5.4–8.3</td>
<td>5.0–9.0</td>
<td>5.0–9.0</td>
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<tr>
<td>Optimum</td>
<td>7.5</td>
<td>7.0–8.0</td>
<td>7.0</td>
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<td>NaCl for growth (g l&lt;sup&gt;−1&lt;/sup&gt;)</td>
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<tr>
<td>Range</td>
<td>5–60</td>
<td>5–100</td>
<td>5–80</td>
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<tr>
<td>Optimum</td>
<td>20–25</td>
<td>30</td>
<td>20</td>
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<tr>
<td>Oxidase</td>
<td>–</td>
<td>–</td>
<td>ND</td>
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<tr>
<td>Catalase</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td><strong>Substrates for growth</strong></td>
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<tr>
<td>Casein</td>
<td>+</td>
<td>ND</td>
<td>–</td>
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<tr>
<td>Casamino acid</td>
<td>+</td>
<td>ND</td>
<td>–</td>
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<tr>
<td>Glutamine</td>
<td>+</td>
<td>ND</td>
<td>–</td>
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<tr>
<td>Starch</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Glucose</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Galactose</td>
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<tr>
<td>Fructose</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Xylose</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>43.2</td>
<td>43.5</td>
<td>44.9</td>
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<td>Major fatty acids (%)</td>
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<tr>
<td>anteiso-C&lt;sub&gt;15 : 0&lt;/sub&gt;</td>
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<td>16.6</td>
<td>8.8</td>
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<tr>
<td>branched-C&lt;sub&gt;17 : 1&lt;/sub&gt;</td>
<td>12.0</td>
<td>–</td>
<td>–</td>
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<tr>
<td>C&lt;sub&gt;15 : 1&lt;/sub&gt;</td>
<td>7.2</td>
<td>6.6</td>
<td>–</td>
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<td>cyclo-C&lt;sub&gt;17 : 0&lt;/sub&gt;</td>
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<td>5.7</td>
<td>10.0</td>
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<td>iso-C&lt;sub&gt;16 : 0&lt;/sub&gt; 3-OH</td>
<td>5.4</td>
<td>–</td>
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<td>iso-C&lt;sub&gt;17 : 0&lt;/sub&gt; 3-OH</td>
<td>–</td>
<td>9.3</td>
<td>–</td>
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<td>C&lt;sub&gt;16 : 0&lt;/sub&gt;</td>
<td>–</td>
<td>6.1</td>
<td>12.4</td>
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<td>anteiso-C&lt;sub&gt;17 : 0&lt;/sub&gt; 3-OH</td>
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<td>5.8</td>
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<td>C&lt;sub&gt;15 : 0&lt;/sub&gt;</td>
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<td>C&lt;sub&gt;18 : 0&lt;/sub&gt; 3-OH</td>
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<td>5.1</td>
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</table>
et al., 2011), were grown with JCM medium 512 at 22 °C and in MB at 30 °C, respectively. Fatty acids of the isolate and its closest phylogenetic neighbours were obtained from cells by saponification, methylation and extraction according to the Sherlock Microbial Identification System (MIDI, 1999). Fatty acid compositions were determined using a Finnigan TRACE DSQ GC-MS system (Thermo Fisher Scientific) equipped with a DB-5 column (J&W Scientific) with a helium flow of 1.5 ml min⁻¹ and an oven temperature program increasing from 140 °C (5 min) to 280 °C (5 min) at 4 °C min⁻¹. The major cellular fatty acids were found to be anteiso-C₁₅:₀ (17.5 %), iso-C₁₅:₀ (16.7 %), branched-C₁₇:₁ (12.0 %), C₁₅:₀ (7.2 %), C₁₇:₀ cyclo (7.1 %) and iso-C₁₆:₀ 3-OH (5.4 %) (Table 1 and Table S1). The predominance of branched saturated C₁₅ acids in the cellular fatty acid composition is common in the Bacteroidetes. The major cellular fatty acid composition of the isolate was more like that of S. elliptica LMG 25367T than that of P. bellariivorans JCM 13498T. Nevertheless, the major fatty acid composition of the isolate was characterized specifically by the predominance of branched-C₁₇:₁. In addition, in the GC-MS analysis, not only fatty acid methyl esters but also aliphatic aldehydes such as iso-C₁₄:₀, iso-C₁₅:₀ and anteiso-C₁₅:₀ were detected in strain JAM-BA0302T, and some other aliphatic aldehydes were found in P. bellariivorans JCM 13498T and S. elliptica LMG 25367T (Table S1). These aliphatic aldehydes are probably derived from the potential sphingolipid components, although the presence of the sphingolipids was not identified by the polar lipid analysis using two-dimensional TLC, as will be discussed.

The isoprenoid quinones and polar lipids of the isolate and its closest relative, S. elliptica LMG 25367T, were extracted according to the procedures described by Minnikin et al. (1984). Isoprenoid quinones were purified on TLC and analysed by reversed-phase HPLC according to the method described by Komagata & Suzuki (1987). Polar lipids were extracted using the procedures described by Minnikin et al. (1984) and identified by two-dimensional TLC followed by spraying with the appropriate detection reagents (Minnikin et al., 1984; Komagata & Suzuki, 1987). The major isoprenoid quinone of strain JAM-BA0302T and S. elliptica LMG 25367T was menaquinone-7 (MK-7). Polar lipids analysis showed that both strain JAM-BA0302T and S. elliptica LMG 25367T had phosphatidylethanolamine, two glycolipids and two unknown lipids as the most-abundant polar lipids (Fig. S2). However, the comparison of two-dimensional chromatograms of polar lipid components revealed the significant different composition in between strain JAM-BA0302T and S. elliptica LMG 25367T. Several phospholipids (PL1, PL2, PL3 and PL4), glycolipids (GL3 and GL4) and unknown lipids (L4 and L5) were found only in strain JAM-BA0302T, while some lipid components (GL5, GL6 and a ninhydrin-reaction-positive lipid) were observed only in S. elliptica LMG 25367T (Fig. S2).

Genomic DNA of strain JAM-BA0302T and S. elliptica LMG 25367T was prepared as described by Marmur & Doty (1962). The G+C content was determined by direct analysis of deoxyribonucleotides by HPLC (Tamaoka & Komagata, 1984). The G+C content of the isolate was 43.2 mol%, which was similar to those of other members of the Bacteroidetes (Irgens, 1977; Holmes et al., 2007; Qu et al., 2009; 2011) (Table 1).

The 16S rRNA gene was amplified by PCR using the primers Bac 27F and 1492R (DeLong, 1992; Lane, 1985) as previously described (Takai et al., 2001). A nearly complete sequence (1442 bp) of the 16S rRNA gene was obtained from strain JAM-BA0302T by direct sequencing by both strands using the dideoxynucleotide chain-termination method with a DNA sequencer MegaBACE 1000 (GE Healthcare UK). Using BLAST, the 16S rRNA gene sequence was found to be most closely related to the sequence of a subsurface Bacteroidetes strain, Kimo37, which was isolated from mineral deposits on the borehole cap associated with the deep crustal fluids in the Juan de Fuca Ridge flank (Nakagawa et al., 2006) (99.6 % 16S rRNA gene sequence similarity) and to strain G13a-B from the Indian Continental Shelf (Parkes et al., 2009) (99.7 %). These Bacteroidetes strains have been isolated but their physiological and taxonomic characteristics have been not yet characterized. On the other hand, of the species validly described within the Bacteroidetes, strain JAM-BA0302T showed the highest 16S rRNA gene sequence similarity (95.5 %) with S. elliptica DQHS-4T and the isolate was only distantly related to other members of phylum Bacteroides; 91.5 % with M. glaucopis strain V₁ (Irgens, 1977) (the type strain is now unavailable in public culture collections) and 89.2 % with P. bellariivorans F₂ (Holmes et al., 2007). The nearly complete sequence was manually aligned according to the 16S rRNA secondary structure using ARB (Ludwig et al., 2004). Phylogenetic analyses were inferred with the neighbour-joining and maximum-likelihood methods using MEGA version 5.05 (Tamura et al., 2011). The neighbour-joining tree (Saitou and Nei, 1987) was constructed using the Jukes–Cantor model. The maximum-likelihood tree (Felsenstein, 1981) was drawn using starting default parameters. The cut-off value of columns was 90 % and 1452 positions were used. Bootstrap confidence was calculated using 1000 replications for both methods. Phylogenetic trees were plotted using NJPlot software. The phylogenetic trees indicated that the isolate and the yet-to-be-described Bacteroidetes strains were phylogenetically associated with S. elliptica DQHS-4T and could belong to the genus Sunxiuqinia (Fig. 2).

DNA–DNA relatedness between the isolate and S. elliptica LMG 25367T was examined by DNA–DNA hybridization, which was fluorometrically performed at 42 °C for 3 h according to the method of Ezaki et al. (1989). DNA–DNA relatedness between the isolate and S. elliptica LMG 25367T was <11 %. This low value indicated that the isolate could be genetically differentiated from S. elliptica at the species level. Strain JAM-BA0302T represents one of the most predominant cultivated aerobic heterotrophic populations (~10⁶
colony forming units g⁻¹) in a methane-hydrate-bearing layer of sediment at a depth of 247.1 m below the seafloor in the deep sea (water depth 1180 m) off the Shimokita Peninsula (Kobayashi et al., 2008). Its 16S rRNA gene sequence was most closely related with sequences of anaerobic Bacteroidetes strains isolated from different subseafloor sedimentary environments, such as the Indian Continental Shelf (strain G13a-B) (Parkes et al., 2009) and the mineral deposits associated with the deep crustal fluids in the Juan de Fuca Ridge flank (strain Kimo37) (Nakagawa et al., 2006). Thus, this group within the Bacteroidetes may represent one of the significant organoheterotrophic microbial components living in global subseafloor sediments, particularly in the relatively organic-compounds-rich continental margins.

This is the first description of a member of the Bacteroidetes isolated from the deep subseafloor sedimentary biosphere. 16S rRNA gene sequence analysis indicated that the isolate and its subseafloor relatives are closely related with S. elliptica DQHS-4T, which was isolated from sediment of a sea cucumber culture pond on the east coast of China (Fig. 2) (Qu et al., 2011). Other moderately related members of the Bacteroidetes are M. glaucopis V1 from an anaerobic digester of a German waste treatment plant (Irgens, 1977) and P. bellariivorans F2T from marine sediment at Boston Harbor, USA (Holmes et al., 2007). Most of the chemotaxonomic features of strain JAM-BA0302T and S. elliptica are quite similar, although the abundance of several fatty acids and polar lipids differ between them. However, the cellular morphology and capability of anaerobic fermentative growth of strain JAM-BA0302T, in addition to the difference in requirements for growth, are significant physiological properties that differentiate strain JAM-BA0302T from S. elliptica (Table 1). In addition, the low DNA–DNA relatedness (<11 %) and 16S rRNA gene sequence similarity (95.5 %) are far below the recommended and accepted criteria for DNA–DNA relatedness (70 %) and 16S rRNA gene sequence similarity (97 %) for differentiating bacterial species (Stackebrandt & Goebel, 1994; Stackebrandt et al., 2002; Wayne et al., 1987). Thus, on the basis of phylogenetic and physiological properties, the isolate belongs to the genus Sunxiuqinia but can be distinguished as a representative of a novel species, for which we propose the name Sunxiuqinia faeciviva sp. nov.

**Description of Sunxiuqinia faeciviva sp. nov.**

*Sunxiuqinia faeciviva* (fae.ci.vi'va. L. n. faex fecis sediment; L. adj. vivus -a -um living; N.L. fem. adj. faeciviva living in sediment).

Cells occur singly as Gram-negative, straight to curved rods with peritrichous fimbriae-like structures and no flagella and are motile by gliding (4–28 × 0.5–0.7 μm in the exponential growth phase). Cells become spherical in the stationary growth phase. Facultatively anaerobic. Grows at 4–37 °C (optimum 30 °C), at pH 5.4–8.3 (optimum pH 7.5) and with 5–60 g NaCl l⁻¹ (optimum 20–25 g l⁻¹). Piezotolerant up to 25 MPa and 10 MPa under aerobic and anaerobic conditions, respectively. Organoheterotrophic with aerobic respiration and anaerobic fermentation of complex proteinaceous

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**Fig. 2.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain JAM-BA0302T and representative members of the phylum Bacteroidetes. Bootstrap values (>70 %) based on 1000 replicates are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered using maximum parsimony and maximum likelihood (>60 % bootstrap support, 1000 and 250 replicates, respectively). Bar, 2 substitutions per 100 nt.
substrates. The major cellular fatty acids are anteiso-C_{15:0},
iso-C_{15:0}, branched-C_{17:1}, C_{15:1}, C_{17:0} cyclo and iso-C_{16:0} 3-OH.
The major isoprenoid quinone is menaquinone-7 and
the major polar lipids are phosphatidylethanolamine, several
glycolipids and other unknown lipids.

The type strain is JAM-BA0302^T (=JCM 15547^T =NCIMB 14481^T),
isolated from a deep subseafloor sediment at a
depth of 247.1 m below the seafloor (water depth 1180 m)
off the Shimokita Peninsula of Japan, north-western
Pacific Ocean. The DNA G+C content of the type strain
is 43.2 mol% (HPLC).

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