**Methyloprofundus sedimenti** gen. nov., sp. nov., an obligate methanotroph from ocean sediment belonging to the ‘deep sea-1’ clade of marine methanotrophs

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We report the isolation and growth characteristics of a gammaproteobacterial methane-oxidizing bacterium (*Methylococccaceae* strain WF1T, ‘whale fall 1’) that shares 98 % 16S rRNA gene sequence identity with uncultivated free-living methanotrophs and the methanotrophic endosymbionts of deep-sea mussels, ≤ 94.6 % 16S rRNA gene sequence identity with species of the genus *Methylobacter* and ≤ 93.6 % 16S rRNA gene sequence identity with species of the genera *Methylomonas* and *Methyllosarcina*. Strain WF1T represents the first cultivar from the ‘deep sea-1’ clade of marine methanotrophs, which includes members that participate in methane oxidation in sediments and the water column in addition to mussel endosymbionts. Cells of strain WF1T were elongated cocci, approximately 1.5 μm in diameter, and occurred singly, in pairs and in clumps. The cell wall was Gram-negative, and stacked intracytoplasmic membranes and storage granules were evident. The genomic DNA G+C content of WF1T was 40.5 mol%, significantly lower than that of currently described cultivars, and the major fatty acids were 16:0, 16:0:9c, 16:1ω9t, 16:1ω8c and 16:2ω9t.14. Growth occurred in liquid media at an optimal temperature of 23 °C, and was dependent on the presence of methane or methanol. Atmospheric nitrogen could serve as the sole nitrogen source for WF1T, a capacity that had not been functionally demonstrated previously in members of *Methylobacter*. On the basis of its unique morphological, physiological and phylogenetic properties, this strain represents the type species within a new genus, and we propose the name *Methyloprofundus sedimenti* gen. nov., sp. nov. The type strain of *Methyloprofundus sedimenti* is WF1T (=LMG 28393T=ATCC BAA-2619T).

The marine methane cycle represents both a source and a sink to the global greenhouse gas inventory, yet relatively few of the micro-organisms that participate in this cycle have been cultivated, making controlled analysis of their role in the mitigation of methane release from the ocean challenging. Cultivated marine methanotrophs include *Methylobacter marinus*, *Methylomarinum vadi*, *Methylobacterium pelagicum* and *Methylomicrobium pelagicum* (Bowman et al., 1993; Hirayama et al., 2013; Kalyuzhnaya et al., 2008; Sieburth et al., 1987). In ocean sediments, assemblages of anaerobic archaea and diverse communities of aerobic bacteria oxidize methane to cellular carbon (reviewed by Reeburgh, 2007). In ocean waters, significantly less methanotrophic diversity has been documented, with only a handful of proteobacterial methane-oxidizing bacteria (MOB) identified as planktonic aerobic methanotrophs (Li et al., 2014; Swan et al., 2011; Tavormina et al., 2008). The gammaproteobacterial MOB clade ‘deep sea-1’ (Lüke & Frenzel, 2011) comprises uncultivated members that participate in methane oxidation in the water column (Hayashi et al., 2007; Li et al., 2014) and sediments (Elsiaed et al., 2004; Redmond et al., 2010). Some members of ‘deep sea-1’ also form endosymbiotic relationships with *Bathymodiolus* mussels (Duperron et al., 2007; Raggi et al., 2013; Spiridonova et al., 2006), thereby facilitating these animals’ life cycles near methane seeps on the cold, dark ocean floor. More recently, *Idas* mussels living near organic falls (e.g. whale falls and wood falls) have also been shown to host ‘deep sea-1’-associated intracellular methanotrophs...
Strain WF1T (‘whale fall 1’) was isolated from marine sediment in Monterey Canyon off the coast of California, USA (36.708° N 122.105° W; 1828 m below sea-level). Surface (0–1 cm) sediment in close proximity to a whale fall (whale 1820, ‘Patrick’; Braby et al., 2007) was collected on 6 June 2011 using the R/V Western Flyer and ROV Doc Ricketts, owned and operated by the Monterey Bay Aquarium Research Institute. This whale fall lies 500 m from a methane cold seep and 23 km from shore. Physicochemical values recorded during sampling included temperature (2.2 °C), salinity (34.6 practical salinity units) and oxygen (166.7 μM; 3.712 ml l⁻¹). Approximately 10 g sediment was placed in a foil-wrapped 120 ml serum bottle sealed with a rubber stopper and crimp cap and incubated at 4 °C without shaking, under laboratory air with added methane and propane each equalling 30% (v/v) of the headspace volume. Propane was initially included to allow for concurrent enrichment of short-chain-hydrocarbon oxidizers (e.g. Redmond et al., 2010), and was excluded from subsequent incubations specifically aimed at methanotroph isolation. After 12 months, approximately 10 μl sediment was inoculated onto solid nitrate mineral salts medium (NMS; ATCC 1306) modified as follows: trace element solution from medium ATCC 1306 was replaced with trace element solution from medium DSM141 (Robb et al., 1995), vitamin solution from medium DSM141 (ibid.; used as a 1000-fold stock) was added and NaCl was added to a final concentration of 0.6 M (3.5%, w/v). This modified NMS was solidified with 2% Bacto agar (Becton Dickinson). Both agar plates and slants (in Balch-style serum tubes and incubated with rocking at 4, 18, 23, 26, 29 and 32 °C. Strain WF1T exhibited doubling times of 52 h at 4 °C, 14 h at 10 °C, 9.5 h at 18 °C, 9 h at 23 °C and 11 h at 26 °C, and did not grow at or above 29 °C. To test the effect of pH on growth, the concentrations of KH₂PO₄ and Na₂HPO₄ were adjusted to vary the pH by 0.5 unit increments (pH 4.5–8.0), while maintaining a constant phosphate concentration. The strain grew at pH 6–8; the optimum pH for growth was 6.5–7.5. To test the effect of salt concentration on growth, the NaCl concentration was adjusted to achieve final concentrations of 0, 0.17, 0.34, 0.51, 0.68, 0.85, 1.02 and 1.2 M (0, 1, 2, 3, 4, 5, 6 and 7%, w/v). The strain required NaCl for growth, and grew at concentrations from 0.17 to 0.68 M, with optimal growth at 0.34 M NaCl (2%, w/v).

Effects of heat and desiccation

To test the ability of strain WF1T to withstand heat, exponentially growing cells were heated to 40, 50, 60, 70 or 80 °C for 10 min, diluted 1:10 into fresh medium, incubated under standard conditions and inspected for growth at 3, 7 and 14 days. The ability of strain WF1T to withstand desiccation was tested as described previously (Whittenbury et al., 1970). Spore formation was tested by
heating a 6-week-old stationary-phase culture to 80 °C for 20 min, diluting 1:100 into fresh medium and incubating under standard conditions, and observed for growth at 3, 7 and 14 days. The strain did not survive any of these treatments, and spore formation was not observed.

**Morphological characteristics, FISH and transmission electron microscopy**

Cells were observed using light microscopy, phase-contrast microscopy, FISH and transmission electron microscopy. For light, phase-contrast and fluorescence microscopy, an Olympus BX51 microscope was used at ×1000 magnification under oil immersion. Phase-contrast was employed to assess motility of wet-mount cells in exponential and stationary-phase cultures. Under light microscopy, cells appeared coccoid or slightly elongated and frequently occurred in pairs and clumps. Under wet-mount phase-contrast microscopy, cells were non-motile and appeared to have capsules. Capsules were confirmed with negative staining (Fig. 1a), and are common among gammaproteobacterial methanotrophs (Bowman et al., 1993). FISH was routinely employed to verify culture purity. For FISH, 0.5 ml late-exponential-phase liquid culture was fixed with 2 % formaldehyde for 2 h at room temperature. 16S rRNA-targeted fluorescently labelled oligonucleotide probes (Integrated DNA Technologies) specific for all bacteria (Fluos-labelled EUB 338 I-III; Amann et al., 1990; Daims et al., 1999) and gammaproteobacterial methanotrophs (Cy3-labelled MetI-444; Lösekann et al., 2007) and 4′,6-diamidino-2-phenylindole (DAPI) were used to assess culture purity. Controls (‘no probe’ and Cy3-labelled NonEUB338; Wallner et al., 1993) were included to examine the possibility of non-specific probe binding. Under fluorescence microscopy (FISH), a single morphological type was observed: elongated coccoid cells that contained intracellular areas resistant to staining, consistent with the presence of storage granules (Fig. 1b).

To prepare cells for transmission electron microscopy, 5 ml late-exponential-phase culture was fixed for 2 h on ice with 2 % glutaraldehyde and then rinsed with 1 × PBS. Cells were washed and resuspended three times in 1 ml buffer A (50 mM sodium cacodylate, pH 7.4) plus 0.6 M NaCl, followed by post-fixation in buffer A plus 1 % OsO4 for 30 min on ice. Cells were then rinsed five times in 1 ml double-distilled water, and stained with 2 % uranyl acetate in water overnight. Cells were dehydrated on ice in a graded ethanol series (20, 50, 70, 90 and 100 %) and infiltrated in 1:1 Durcupan ACM resin/ethanol for 30 min followed by three sequential 60 min 100 % Durcupan steps prior to polymerization at 60 °C for 48 h. Sections (200 nm) were obtained on a Leica ultramicrotome. Cells were imaged on a Zeiss EM 109 transmission electron microscope, operated at 50 kV. Electron microscopy revealed a typical Gram-negative cell wall, intracytoplasmic membrane stacks (∼10–25 layers) and storage granules, probably polyhydroxyalkanoate or polyhydroxybutyrate (Fig. 1c).

**Carbon and nitrogen source utilization**

Methane or methanol was required for growth; methane supported growth at all concentrations tested [5–50 % (v/v) headspace], and methanol supported growth in the absence of methane at all concentrations tested (0.1, 0.2, 0.3, 0.4 and 0.5 %, v/v). Growth of members of the genus Methylo bacter may be inhibited by direct addition of methanol (>0.1 %, v/v) to the medium (pp. 258–259 in Bowman, 2005), making the growth of WF1T under all methanol concentrations tested a distinguishing feature of this strain. Alternative potential carbon sources including glucose, sucrose, acetate, citrate, pyruvate, succinate, formate, formaldehyde, formamide, yeast extract, glycerol, dimethylformamide and ethanol were tested for their ability to
support growth in the absence of methane by supplementing modified NMS with each respective carbon source to 0.1 % (w/v). For these carbon source utilization tests, exponential-phase cells were diluted 1 : 100, incubated under standard conditions and examined for growth after 7, 14 and 25 days. Each of these alternative carbon sources failed to support growth. Alternative potential nitrogen sources including ammonium, Tris, urea, nitrite, yeast extract, glucosamine, trimethylamine, formamide, taurine, thiamine, cysteine, glycine, isoleucine, leucine, lysine, methionine, proline, tryptophan and valine were tested for their ability to support growth in the absence of nitrate by replacing nitrate with each respective nitrogen source in modified NMS at a final concentration of 0.05 % (w/v). Atmospheric nitrogen was tested for its ability to serve as a sole nitrogen source by preparing medium without any added nitrogen sources, adding exponential-phase cells at a 1 : 100 dilution and passing three times in this manner to exclude the possibility that trace nitrate in the original inoculum was serving as the nitrogen source. For these nitrogen source utilization tests, exponential-phase cells were diluted 1 : 100, incubated under standard conditions and examined for growth after 7, 14 and 25 days. Atmospheric nitrogen, nitrate, ammonium, urea, yeast extract, glucosamine, leucine, lysine and, to a lesser degree, cysteine were able to serve as nitrogen sources. Although genomes of members of the genus Methylobacter contain genes for nitrogen fixation, a direct demonstration of nitrogen fixation from a member of Methylobacter has not been reported (Bowman, 2005), revealing a unique potential of the ‘deep sea-1’ clade relative to its closest cultured relatives. The utilization by strain WF1T of lysine and leucine as nitrogen sources further distinguishes this strain from previously described species of the genera Methylo bacter and Methylocrasis (Bowman et al., 1993). A requirement for vitamin supplementation was tested by preparing NMS medium without added vitamins. Exponential-phase cells were added to this medium at a 1 : 100 dilution and passed three times in this manner to exclude the possibility that trace vitamins in the original inoculum were promoting growth. Strain WF1T initially grew in vitamin-free medium, but growth diminished in subsequent passages, suggesting that growth in culture is promoted by the presence of vitamins.

Fatty acid extraction and analysis

Frozen cell pellets were lyophilized and pre-weighed prior to extraction. Lipid extraction and trans-esterification to fatty acid methyl esters (FAMEs) were done simultaneously at room temperature with 1 ml hexane and 2 ml 0.5 M NaOH in anhydrous methanol. The reaction was neutralized after 10 min with 70 μl glacial acetic acid and the FAMEs were subsequently extracted with 2 ml water and 2 ml hexane (Griffiths et al., 2010). A solution of tricosane (40 ng μl⁻¹) was used as an internal standard for extraction efficiency and quantification and was added prior to trans-esterification. Double bond positions were identified from 3-pyridylcarbinol esters of fatty acids (Christie & Han, 2010). The reaction catalyst was prepared in a N₂-flushed vial by combining 200 μl 3-(hydroxymethyl)pyridine and 100 μl 1 M t-butoxide in tetrahydrofuran. FAMEs were dissolved in dry dichloromethane and then added by syringe to the reaction catalyst. The mixture was heated at 40 °C for 20 min. After cooling to room temperature, the 3-pyridylcarbinol esters were extracted with water and hexane, dried over Na₂SO₄ and concentrated prior to analysis.

FAMEs and 3-pyridylcarbinol esters of fatty acids were analysed by gas chromatography-mass spectrometry (GC-MS) on a Thermo-Scientific Trace-DSQ with a ZB-5ms column (30 m × 0.25 mm, 0.25 μm i.d.) using a programmed temperature vaporization injector operated in splitless mode. The oven temperature program was 100 to 320 °C (held for 20 min) at 6 °C min⁻¹. Quantification was done using a flame-ionization detector. Peak areas were determined using Thermo Xcalibur version 2.1. Compound identification was based on comparison of retention time and mass spectra to known standards as well as to the NIST mass spectra library version 2.0.

The cellular fatty acid profile of strain WF1T, containing primarily C₁₆ fatty acids, is consistent with those of other type I methanotrophic bacteria (Bowman et al., 1991, 1993); however, it is differentiated by the presence of 16 : 2 fatty acids (Bodelier et al., 2009). Three C₁₆ : 1 fatty acids were present in similar proportions in strain WF1T: 16 : 1o9c (28.8 %), 16 : 1o9t (26.9 %) and 16 : 1o8c (22.3 %). Additionally, 16 : 0 (15 %) and 16 : 2o9,14 (7.1 %) were detected in fatty acid extracts. A summary of the distinguishing features of strain WF1T is provided in Table 1.

Sequence analysis

DNA was extracted from 200 ml of a late-exponential-phase culture following established methods (Schmidt et al., 1991) and further purified to a level suitable for PCR and sequencing (Saunders & Burke, 1990). PCR amplifications were performed using established primers and methods for the 16S rRNA gene, pmoA and the methanol dehydrogenase gene mxaF (Lane, 1991; McDonald & Murrell, 1997; Tavormina et al., 2008, 2010). PCR amplicons were sequenced using a commercial service (Laragen, Culver City, CA, USA) and BigDye chemistry. For genome sequencing, purified DNA (10 μg) was sent to SeqMatic (Union City, CA, USA) for Illumina sequencing on the MiSeq platform. Data were uploaded to http://rast.nmpdr.org/ for automatic annotation.

A total of 4 065 599 bp on 684 contiguous DNA fragments were recovered in the partial genome sequence of strain WF1T. The genomic G+C content was 40.5 mol%, significantly lower than those of previously described methanotrophic cultivars, including Methylobacter luteus (49 mol%), the type species of the genus Methylobacter, and Methylo bacter psychrophilus (46 mol%), as well as the Antarctic methanotroph Methylophaera hansonii (43.5 mol%).
Table 1. Characteristics of strain WF1\textsuperscript{T} in comparison with related methanotrophic genera

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>6</th>
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<tr>
<td>Temperature for growth (°C)</td>
<td>Range</td>
<td>4–26</td>
<td>3.5–37</td>
<td>5–37</td>
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<td>Range</td>
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<td>5–9</td>
<td>3.8–10.5</td>
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<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>6–8</td>
<td>ND</td>
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<td>Salinity range (%, w/v)</td>
<td>Range</td>
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<td>0–2.5</td>
<td>0–8.8</td>
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<td>Cocci, ellipsoids, rods</td>
<td>Cocci, ellipsoids, rods</td>
<td>Short rods</td>
<td>Cocci, rods</td>
<td>Cocci, ellipsoids, rods</td>
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<td>Motility</td>
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<td>Major PLFAs (%)\textsuperscript{a}</td>
<td>16:0 (15), 16:1ω9c (22), 16:1ω9t (27), 16:2ω9t,14 (7)</td>
<td>14:0 (7–11), 16:0 (7–9), 16:1ω8 (0–35), 16:1ω7 (23–38), 16:1ω6c (4–6), 16:1ω5c (6–8), 16:1ω5t (10–26)</td>
<td>14:0 (19–25), 16:0 (5–9), 16:1ω8c (19–41), 16:1ω7c (7–15), 16:1ω5c (2–6), 16:1ω5t (8–16)</td>
<td>16:0 (12–18), 16:1ω8c (13–19), 16:1ω7c (36–51), 16:1ω6c (5–7), 16:1ω5t (10–30), 18:1ω7c (0–27)</td>
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<td>–</td>
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<td>C. assimilation pathway</td>
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<td>Presence of nifH\textsuperscript{b}</td>
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<tr>
<td>Nitrogen fixation (function)\textsuperscript{b}</td>
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<td>+</td>
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<td>DNA G + C content (mol%)</td>
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<td>46–55</td>
<td>48.5–59.0</td>
<td>46–51</td>
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<td>49.3</td>
<td>43.5–45.9</td>
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<td>Maximum 16S rRNA gene sequence identity to strain WF1\textsuperscript{T} (BLAST) (%)\textsuperscript{c}</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>95</td>
<td>94</td>
<td>93</td>
<td>92</td>
<td>94</td>
<td>92</td>
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<tr>
<td>Genomic ANI to strain WF1\textsuperscript{T} (%)\textsuperscript{c}</td>
<td>79.3–80.5</td>
<td>81.5</td>
<td>81.7–82.7</td>
<td>NG</td>
<td>NA</td>
<td>NG</td>
<td>80.3</td>
<td>NG</td>
<td>80.3</td>
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</table>

\*PLFAs representing more than 5% of the total cellular pool are shown. Data for PLFA abundances for reference taxa were taken from Bowman et al. (1993, 1995, 1997), Hirayama et al. (2013), Iguchi et al. (2011), Rahalkar et al. (2007) and Wartiainen et al. (2006).

\textsuperscript{b}Presence of nifH gene, or assessment of function either by acetylene reduction assay or by growth on atmospheric nitrogen. In addition to articles describing specific methanotrophic taxa, see Auman et al. (2001).

\textsuperscript{c}Two-way average nucleotide identity (ANI) (http://enve-omics.ce.gatech.edu/ani/). NA, Insufficient hits for two-way ANI calculation; NG, no genome data available.
Preliminary annotation of the partial genome of strain WF1T revealed genetic capacity common to many protobacterial cultivars, including single copies of genes for 16S rRNA, 23S rRNA and pmoCAB. pxm genes and genes encoding soluble methane monoxygenase (sMMO) were not present in the partial genome of strain WF1T. Several genes encoding enzymes relevant to the serine pathway of formaldehyde assimilation were identified; however, a complete serine pathway could not be constructed from the partial genome. Likewise, the Calvin–Benson–Bassham cycle could not be constructed. Genes encoding a complete ribulose monophosphate (RuMP) pathway of carbon assimilation were identified, as were genes for nitrogen fixation, capsule formation and glyco- gen, polyphosphate and polyhydroxyalkanoate (polyhydroxybutyrate) storage. Genes predicted to encode urease and a leucine-specific transporter were also identified in the genome, which may pertain to the ability of strain WF1T to utilize urea and leucine as nitrogen sources. Genes predicted to encode cytochrome c oxidase and catalase were also present, and a complete ubiquinone biosynthetic pathway was identified.

Phylogenetic analysis

To reconstruct a phylogenetic tree for the 16S rRNA gene, sequences from type species of the family Methylococcaceae were used to infer a tree by maximum-likelihood using the PhyML package (Guindon et al., 2010) and the HKY evolutionary model in the software program ARB version 5.5 (http://www.arb-home.de/; Ludwig et al., 2004). Nearly full-length 16S rRNA gene sequences were used, where the shortest sequence was 1396 bases (GenBank accession no. GU584415). The sequences were aligned in the SSURef-111-SILVA-NR database (http://www.arb-silva.de/; Quast et al., 2013) and masked using the bacterial filter provided. Clone 2E-055 (GenBank accession no. FJ981085; 683 bases) was inserted into the tree using the parsimony insertion algorithm in ARB. The reliability of the tree was estimated by bootstrapping in software program Geneious version 7.1.7 (http://www.geneious.com/) using PhyML maximum-likelihood, the HKY model and 1000 replicates. Sequences that served as an outgroup (not shown) included those of Methylocapsa acidiphila B2T (GenBank accession no. AJ278726), Methylocystis parvus OBBP T (Y18945) and Methylosinus trichosporium OB3bT (ADVE01000118). To reconstruct a phylogenetic tree for the pMMO A-subunit, the pmoA gene sequence of strain WF1T was translated in silico to protein sequence, aligned with pMMO A sequences from cultured methanotrophs within the family Methylococcaceae with validly published names and relevant environmental sequences using MUSCLE (http://www.phylogeny.fr; Dereeper et al., 2008) and trimmed to residues 63–189 (positions according to the sequence of Methylococcus capsulatus Bath). The resulting alignment was analysed via the neighbour-joining and maximum-likelihood treeing methods, with the AMO-A sequence of ‘Nitrosomonas cryotolerans’ ATCC 49181 (GenBank accession no. AF314753) as the outgroup.

The 16S rRNA gene sequence from strain WF1T was 97–99% identical to multiple 16S rRNA gene sequences assigned to uncultured methanotrophic endosymbionts of bathydemodiolin mussels (Duperron et al., 2007; Raggi et al., 2013; Spiridonova et al., 2006) and sequences recovered from the marine water column and marine sediments (Elsaied et al., 2004; Li et al., 2014; Redmond et al., 2010; Schauer et al., 2011; Schmidtova et al., 2009). These sequences formed a clade separate from cultured methanotrophic genera. When compared to existing cultivars, the 16S rRNA gene sequence of strain WF1T was most closely related to those of members of Methylobacter (≤94.6% sequence identity), Methylosarcina (≤93.6% sequence identity) and Methylosomas (93.6% sequence identity) (Fig. 2a, Table 1). Phylogenetic analysis of the pMMO A protein revealed similar patterns of relatedness between strain WF1T and its phylogenetic divergence from established cultivars, and the monophyletic relationship with the ‘deep sea-1’ clade of uncultured microorganisms, which includes sediment-, water column- and mussel-hosted members, we propose the name Methyloprofundus sedimenti gen. nov., sp. nov.

Description of Methyloprofundus gen. nov.

Methyloprofundus [Me.thy’lo.pro.fun’ dus] N.L. pref. methyl- pertaining to the methyl radical, from N.L. n. methylum (from Fr. adj. méthode) the methyl group; L. masc. adj. profundus of the deep; N.L. masc. n. Methyloprofundus a methyl-using bacterium from the deep sea.

Deep-sea marine bacteria with Gram-negative cell wall. Cells are coccolid to slightly elongated, frequently occurring in pairs, and stacked intracytoplasmic membranes are evident. Utilize methane and methanol as sole carbon sources. Possess pMMO and assimilate carbon via the ribulose monophosphate pathway. Do not possess sMMO. The genus is a member of the family Methylococcaceae. The type species is Methyloprofundus sedimenti.
Fig. 2. (a) 16S rRNA gene phylogeny showing the placement of strain WF1T within the order Methylococcales of the class Gammaproteobacteria. The tree was inferred with maximum-likelihood, the HKY evolutionary model and 1000 bootstraps. Bootstrap support of 60% or greater is indicated by open circles and 80% or greater by filled circles. Bar, 0.10 substitutions per site. (b) pMMO-A phylogeny. Neighbour-joining tree based on predicted protein sequences (residues 63–189 from the sequence of Methylococcus capsulatus Bath) from strain WF1T and cultured type I methanotrophs. The branch labelled 'deep sea-1' includes strain WF1T and representative uncultured sequences from the water column [OPU2 (Hayashi et al., 2007); Guaymas Basin metatranscriptome c15185 (Li et al., 2014)], sediment (Methane-SIP; Redmond et al., 2010) and endosymbionts of mussels (endosymbiont of B. childressi; Duperron et al., 2007). Bootstrap support of 60% or greater is indicated by open circles and 80% or greater by solid circles (1000 replicates). Bar, 0.10 substitutions per site.
Description of Methyloprofundus sedimenti sp. nov.

Methyloprofundus sedimenti (se.di.men’ti. N.L. masc. adj. sedimenti of sediment).

In addition to the characteristics of the genus, the following traits are observed. Cells are 1–1.5 μm in diameter and non-motile. Requires NaCl for growth, with optimal NaCl concentration of 2%. Optimal growth temperature is 18–23 °C; growth does not occur at or above 29 °C. Utilizes nitrate, ammonium, yeast extract, glucosamine, urea, cysteine, leucine, lysine and atmospheric nitrogen as sole nitrogen sources. Predominant fatty acids are 16:0, 16:1o9c, 16:1o9t, 16:1o8c and 16:2o914. Does not form colonies on NMS solid medium.

Strain WF1T (=LMG 28393T=ATCC BAA-2619T) is the type strain, isolated from marine sediment near a whale fall in Monterey Canyon, California. The G+C content of genomic DNA of the type strain is 40.5 mol%.

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