Methyloprofundus sedimenti gen. nov., sp. nov., an obligate methanotroph from ocean sediment
belonging to the Deep Sea 1 clade of marine methanotrophs

Running title: Methyloprofundus sedimenti gen. nov. sp. nov.

New Taxon, subsection Proteobacteria

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The Genbank accession numbers for the 16S rRNA, pmoA, and mxAF genes from this taxon are KF484906-484908.

Abbreviations: Whale Fall 1:WF1, methane oxidizing bacteria: MOB, Particulate methane monooxygenase: pmo; optical density: OD, 4',6-diamidino-2-phenylindole: DAPI, nitrate mineral salts: NMS, fatty acid methyl esters: FAMES.
Summary

We report the isolation and growth characteristics of a gammaproteobacterial methane-oxidizing bacterium (*Methylococcaceae* strain WF1, “whale fall 1”) that shares 98% 16S rRNA identity with uncultivated free-living methanotrophs and the methanotrophic endosymbionts of deep sea mussels, 94.6% 16S rRNA identity with *Methylobacter* species, and 93.6% 16S rRNA identity with *Methylomonas* and *Methylosarcina* species. Strain WF1 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. WF1 cells were elongated cocci approximately 1.5 µm in diameter, and occurred singly, in pairs and clumps. The cell wall was Gram negative, and stacked intracytoplasmic membranes and storage granules were evident. The genomic GC content of WF1 was 40.5%, significantly lower than currently described cultivars, and the major fatty acids were 16:0, 16:1 ω9c, 16:1 ω9t, 16:1 ω8c and 16:2 ω9, 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 WF1, a capacity that had not been functionally demonstrated in members of *Methylobacter*. On the basis of unique morphological, physiological, and phylogenetic properties this strain represents the type species within a new genus, and we propose the name *Methyloprofundus sedimenti* (type strain WF1 = BCCM LMG 28393 = ATCC BAA-2619).
The marine methane cycle represents both a source and a sink to the global greenhouse gas inventory, yet relatively few of the microorganisms participating 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*, *Methylovorans vadi*, *Methylomicrobium pelagicum* and *Methylomicrobium japonense* (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 in 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., 2013; Swan et al., 2011; Tavormina et al., 2008). The gammaproteobacterial MOB clade “Deep Sea 1” (Luke and Frenzel, 2011) is comprised of uncultivated members that participate in water column (Hayashi et al., 2007; Li et al., 2013) and sediment (Elsaied et al., 2004; Redmond et al., 2010) methane oxidation. 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 (reviewed in Duperron et al., 2013; Petersen and Dubilier, 2009). Thus, the Deep Sea 1 clade is interesting not only for its role in the marine methane cycle as it pertains to both sediments and the water column, but also as a potential window into events that lead to endosymbiont acquisition in animals. Here we report the cultivation and initial characterization of the first representative of this clade.

*Methylococcaceae* WF1 (“whale fall 1”) was isolated from marine sediment in Monterey Canyon, California (36.708N, -122.105W; 1828 mbsl). Surface (0-1 cm) sediment in close proximity to a whale fall (whale 1820, “Patrick” (Braby et al., 2006)) was collected on June 6, 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. Physico-chemical values recorded during sampling included temperature (2.2°C), salinity (34.6 PSU), and oxygen (166.7 µM; 3.712 ml/l). Approximately 10 grams of sediment were 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 equaling 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 of sediment was inoculated onto solid nitrate mineral salts medium (NMS, ATCC 1306) modified as follows: Trace element solution from media 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 sodium chloride (NaCl) was added to a final concentration of 0.6M (3.5% w/v). This modified NMS was solidified with 2% Bacto agar (Becton, Dickinson, and Company; Franklin Lakes, New Jersey). Both agar plates and slants (in Balch-style serum tubes) were inoculated for single colonies and placed under lab air with added methane (30% v/v of the headspace). Plates and slants were incubated at 10 and 23°C and examined periodically for growth, and the original enrichment bottle was maintained at 4°C.

Four weeks after inoculation of plates and slants, sixty individual colonies were probed molecularly for the gene central to methane metabolism in MOB, particulate methane monoxygenase (pmoA), and four colonies tested positive for identical pmoA sequences. These four colonies were passaged on agar under methane several times, but growth diminished with each passage, suggestive of potential contamination. One isolate was transferred into liquid modified NMS media, where it was successfully passaged. In the weeks following transition to liquid media, a contaminating species was identified as a member of Ahrensia (Uchino et al., 1998) on the basis of microscopy and 16S rRNA gene
sequence (Lane, 1991). Dilution to extinction in liquid media, coupled with a reduction of oxygen concentration in the headspace (50% N₂, 30% CH₄, 20% lab air) promoted methanotroph growth relative to *Ahrensia*, and after several months of successive passaging, no contaminating species were detected. The culture was considered pure when general 16S rRNA primers exclusively amplified the methanotroph 16S rRNA gene, when methane-free incubations supplemented with 0.1% (w/v) yeast extract did not promote any growth, and when all cells in the culture hybridized to the gammaproteobacterial methanotroph-specific fluorescence *in situ* hybridization (FISH) probe MetI-444 (Losekann et al., 2007). Strain *Methylococcaceae* WF1 was stored at -80°C following recent guidelines which promote better revival of methanotroph strains (Hoefman et al., 2012).

Unless otherwise noted, *Methylococcaceae* WF1 was grown in modified liquid NMS medium under 50% N₂, 30% CH₄, 20% lab air, and tubes were incubated at 10°C with rocking. Under these growth conditions, the strain formed a uniformly distributed culture and attained an optical density at 450 nm (OD₄₅₀) of 0.3; direct cell counts indicates that this represents approximately 5 x 10⁷ cells per ml. In the absence of rocking or shaking, cells settled to the bottom of the tube and were easily redistributed into solution via swirling. There was no pellicle formation.

*Physiological properties*

All physiological testing was performed by adjusting the standard medium and diluting log phase cells 100-fold into these media. Growth was monitored as a change in OD₄₅₀. To test the effect of temperature on growth, triplicate cultures of strain WF1 were inoculated in 30 ml Balch-style serum tubes and incubated with rocking at the following temperatures: 4°C, 10°C, 18°C, 23°C, 26°C, 29°C and 32°C. Strain *Methylococcaceae* WF1 exhibited a doubling time of 52 hours at 4°C, 14 hours at 10°C, 9.5 hours at 18°C, 9h at 23°C, 11 hours 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 value by 0.5 unit
increments (4.5 to 8.0), while maintaining a constant phosphate concentration. The strain grew at pH values between 6 and 8; pH optimum for growth was 6.5 – 7.5. To test the effect of salt concentration on growth, NaCl 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 – 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 WF1 to withstand heat, logarithmically growing cells were heated to 40, 50, 60, 70, and 80°C for ten minutes, diluted 1:10 into fresh medium, incubated under standard conditions, and inspected for growth at 3, 7, and 14 days. The ability of strain WF1 to withstand desiccation was tested as described previously (Whittenbury et al., 1970). Spore formation was tested by heating a six week old stationary phase culture to 80°C for 20 minutes, diluting 1:100 into fresh media, 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, fluorescence in situ hybridization (FISH) and transmission electron microscopy (TEM)

Cells were observed using light microscopy, phase-contrast microscopy, fluorescence in situ hybridization (FISH), and transmission electron microscopy (TEM). For light, phase contrast, and fluorescence microscopy, an Olympus BX51 microscope at 1,000X magnification under oil immersion was used. Phase contrast was employed to assess motility of wet-mount cells in log 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 of late log phase liquid culture was fixed with 2% formaldehyde for 2 hours at room temperature. 16S rRNA-targeted fluorescently-labeled oligonucleotide probes (Integrated DNA Technologies, Coralville, Iowa) 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, (Losekann et al., 2007) and 4',6-diamidino-2-phenylindole (DAPI) were used to assess culture purity. Controls (‘no probe’ and Cy3-labeled 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 TEM, 5 ml of late log phase culture were fixed for 2 hours on ice with 2% gluteraldehyde, then rinsed with 1X phosphate buffered saline (PBS). Cells were washed and resuspended in 1 ml buffer A (50mM sodium cacodylate, pH = 7.4) plus 0.6M NaCl three times, followed by post-fixation in in buffer A plus 1% OsO₄ for 30 min on ice. Cells were then rinsed in 1ml ddH₂O a total of 5 times, and stained with 2% uranyl acetate in water overnight. Cells were dehydrated on ice in a graded ethanol series (20, 50, 70, 90, 100%) and infiltrated in 1:1 durecupan ACM resin:ethanol for 30 minutes followed by 3 sequential 60’ 100% durecupan steps prior to polymerization at 60°C for 48 hours. Sections (200nm) were obtained on a Leica ultramicrotome. Cells were imaged on a Zeiss EM 109 transmission electron microscope, operated at 50kV. Electron microscopy revealed a typical Gram-negative cell wall, intracytoplasmic membrane stacks (~10 – 25 layers) and storage granules, likely 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, 0.5% v/v). *Methylobacter* species may be
inhibited by direct methanol addition (>0.1% v/v) to media (Bowman, 2005) pp258-259), making the
growth of WF1 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 the 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, log 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 the 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 the ability to serve as a sole nitrogen source by preparing
medium without any added nitrogen sources, adding log phase cells at a 1:100 dilution, and passaging
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, log 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 *Methylobacter*
species contain genes for nitrogen fixation, a direct demonstration of nitrogen fixation from
*Methylobacter* species 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 WF1 of lysine and leucine as
nitrogen sources further distinguishes this strain from previously described *Methylobacter* and
Methylomonas species (Bowman et al., 1993). A requirement for vitamin supplementation was tested by preparing NMS media without added vitamins. Log phase cells were added to this medium at a 1:100 dilution, and passaged three times in this manner to exclude the possibility that trace vitamins in the original inoculum were promoting growth. Strain WF1 initially grew in vitamin-free media, 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 N 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) 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 and Han, 2010). The reaction catalyst was prepared in an N₂ flushed vial by combining 200 µl 3-(hydroxymethyl)pyridine and 100 µl 1M 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 analyzed by gas chromatography-mass spectrometry (GC-MS) on a Thermo-Scientific Trace-DSQ with a ZB-5ms column (30 m x 0.25 mm, 0.25 µm i.d.) using a programmed temperature vaporization injector operated in splitless mode. The oven temperature program was 100°C to 320°C (held 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 WF1, containing primarily C\textsubscript{16} fatty acids, is consistent with other type I methanotrophic bacteria (Bowman et al., 1991; Bowman et al., 1993); however, it is differentiated by the presence of 16:2 fatty acids (Bodelier et al., 2009). Three C\textsubscript{16:1} fatty acids were present in similar proportions in strain WF1: 16:1 ω 9c (28.8%), 16:1 ω 9t (26.9%), and 16:1 ω 8c (22.3%). Additionally, 16:0 (15%) and 16:2 ω9, 14 (7.1%) were detected in fatty acid extracts. A summary of the distinguishing features of strain WF1 is provided in Table 1.

Sequence analysis

DNA was extracted from 200 ml of a late log phase culture following established methods (Schmidt et al., 1991) and further purified to a level suitable for PCR and sequencing (Saunders and Burke, 1990). PCR amplifications were performed using established primers and methods for the 16S rRNA gene, pmoA sequences, and the methanol dehydrogenase gene mxaF (Lane, 1991; McDonald and Murrell, 1997; Tavormina et al., 2010; Tavormina et al., 2008). PCR amplicons were sequenced using a commercial service and BigDye chemistry (Laragen, Culver City CA) and resulting sequences are deposited in Genbank under accession numbers KF484906-484908. For genomic sequencing, purified DNA (10 micrograms) was sent to SeqMatic (Union City, CA) for Illumina sequencing on the MiSeq platform. Data was 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 WF1 partial genome. The genomic GC content was 40.5%, significantly lower than previously described methanotrophic cultivars including the type species Methylobacter luteus (49% GC) and Methylobacter psychrophilus (46% GC) as well as the Antarctic methanotroph Methylosphaera hansonii (43.5 % GC) (Bowman et al., 1997). Interestingly, low GC content is one of the hallmark features of obligate and
facultative intracellular symbionts and pathogens, possibly resulting from the genetic isolation of small starting populations (Moran et al., 2008). While it is currently unknown whether strain WF1 is capable of establishing an endosymbiotic relationship with mussels, this feature is consistent with phylogenetic placement of this strain within a clade that harbors environmentally acquired endosymbionts of methanotrophic mussels.

Preliminary annotation of the partial genome of strain WF1 reveals genetic capacity common to many gammaproteobacterial cultivars, including single copies of genes for 16S rRNA, 23S rRNA, and pmoCAB. Genes encoding pxm and soluble methane monooxygenase (sMMO) were not present in the partial genome of strain WF1. 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 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 glycogen, 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 WF1 to utilize urea and leucine as nitrogen sources. Genes predicted to encode cytochrome c oxidase and catalase were also present, and a complete ubiquinone (UQ) biosynthetic pathway was identified.

**Phylogenetic analysis**

To construct a phylogenetic tree for the 16S rRNA gene, sequences from validly recognized type species of the *Methylococcaceae* family were inferred 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). Near full-length 16S gene sequences were used, where the shortest sequence was 1396 bases (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 provided bacterial filter. Clone 2E−055 (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 out-group (not shown) included *Methylocapsa acidiphila* (AJ278726), *Methylocystis parvus* (Y18945), and *Methyllosinus trichosporium* str.OB3b (ADVE01000118). To construct a phylogenetic tree for the pMMO-A subunit, the *Methylococcaceae* WF1 *pmoA* gene sequence was translated *in silico* to protein sequence, aligned with pMMO-A sequences from validly recognized cultured methanotrophs within the *Methylococcaceae* family and relevant environmental sequences using MUSCLE (www.phylogeny.fr.org) (Dereeper et al., 2008), and trimmed to residues 63 – 189 (*Methylococcus capsulatus* Bath). The resulting alignment was analyzed via neighbor-joining and maximum likelihood treeing methods, with *Nitrosomonas cryotolerans* AMO-A (AF314753) as the outgroup.

The 16S rRNA gene from strain WF1 was 97-99% identical to multiple 16S rRNA sequences assigned to uncultured methanotrophic endosymbionts of bathymodiolin 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., 2013; Redmond et al., 2010; Schauer et al., 2011; Schmidtova et al., 2009). These sequences formed a monophyletic clade separate from cultured methanotrophic genera. When compared to existing cultivars, the 16S rRNA gene of strain WF1 was most closely related to members of *Methylobacter* (94.6% sequence identity), *Methylosarcina* (93.6% sequence identity), and *Methylomonas* (93.6% sequence identity, Fig. 2a, Table 1). Phylogenetic analysis of the pMMO-A protein revealed similar patterns of relatedness between strain WF1, environmental sequences, and methanotrophic cultivars (Fig. 2b). This relationship was strongly supported for both the
16S rRNA gene sequences (100% bootstrap support, 1,000 replicates) as well as the pMMO-A protein sequences (90% bootstrap support, 1,000 replicates).

Novel nature of Methylococcaceae strain WF1

Because of the unique morphological and physiological features of strain WF1 and its phylogenetic divergence from established cultivars and 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. n. methylum (from the French me’thyle) the methyl group, N.L. prefix methyl- pertaining to the methyl radical; 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 coccoid to slightly elongated frequently occurring in pairs and stacked intracytoplasmic membranes are evident. Utilizes methane and methanol as sole carbon sources. Possesses pMMO and assimilates carbon via the ribulose monophosphate pathway. Does not possess sMMO. Member of family Methylococcaceae. The type species is Methyloprofundus sedimenti.

Description of Methyloprofundus sedimenti, sp. nov.

Methyloprofundus sedimenti (sed.i.men’.ti; N.L. masc. adj. sedimenti: isolated from marine sediment.)

In addition to genus characteristics, the following traits were 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 or atmospheric nitrogen as sole nitrogen source.

Fatty acids are predominantly C16:0 (15%), 16:1 ω 9c (29%), 16:1 ω 9t (27%), 16:1 ω 8c (22%), and C16:2 ω 9, 14 (7%). GC content of genomic DNA is 40.5 mole %. Does not form colonies on NMS solid media.

Strain WF1 is the type strain (= BCCM LMG 28393, ATCC BAA-2619) and was isolated from marine sediment near a whale fall in Monterey Canyon, California.

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References

Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R., Stahl, D.A., 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Applied and environmental microbiology 56, 1919-1925.


Bowman, J.P., Sly, L.I., Nichols, P.D., Hayward, A.C., 1993. Revised taxonomy of the methanotrophs: Description of Methylrobacter gen. nov., Emendation of Methyllococcus, Validation of Methylosinus and Methylcystis species, and a proposal that the family Methylcoccaceae includes only the group I methanotrophs. International journal of systematic and evolutionary microbiology 43, 735-753.


1. PLFAs representing more than 5% of the total cellular pool.

**Not described, v: variable between taxa, NA: insufficient data for two-way ANOVA calculation, NC: no genome data available.**

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**Motility**

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**Salinity range (NaCl)**

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**Temperature range**

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**Characteristics**

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Data for PLFA abundances are taken from (Bowman et al., 1992; Bowman et al., 1995; Bowman et al., 1997; Bowman et al., 1993; Hirayama et al., 1993; Hiroyama et al., 1995; Bowman et al., 1997; Rahalkar et al., 2003; Wartelainen et al., 2006).

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).

Two way average nucleotide identity (http://enveomics.ce.gatech.edu/ani/).
Figure 1. Morphology of strain WF1. (A) Negative stain (1,000X) showing presence of capsules in stationary phase cells. (B) Fluorescence microscopy (1,000X). DAPI (blue), EUB338 I-III (green) and MetI-444 (red) fluorescence in situ hybridization illustrating purity of culture as well as stain-recalcitrant intracellular areas. (C) Thin section of WF1 illustrating typical Type I stacked intracytoplasmic membranes (ICM), storage granules (SG), and a typical Gram negative cell wall (GNCW).

Figure 2. (A) 16S rRNA phylogeny showing the placement of strain WF1 within the Methylococcales order of the γ-subclass of Proteobacteria. Tree was inferred with maximum likelihood, the HKY evolutionary model and 1,000 bootstraps. Bootstrap support of 60 or greater is indicated with open circles; 80 or greater with solid circles. Scale bar represents 0.10 substitutions per site. Quotes indicate an invalid name. (B) pMMO-A phylogeny: Neighbor-joining tree based on predicted protein sequences (residues 63 – 189, Methylococcus capsulatus Bath) from strain Methylococcales WF 1 and cultured Type I methanotrophic genera. The branch labeled “Deep Sea 1” includes strain WF1 and representative uncultured sequences from water column (OPU2, (Hayashi et al., 2007); Guaymas Basin metaT c15185, (Li et al., 2013)), 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 with open circles; 80 or greater with solid circles (1,000 replicates). Scale bar represents 0.10 substitutions per site.