**Methylovulum psychrotolerans** sp. nov., a cold-adapted methanotroph from low-temperature terrestrial environments, and emended description of the genus *Methylovulum*

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Two isolates of aerobic methanotrophic bacteria, strains Sph1T and Sph2, were obtained from cold methane seeps in a floodplain of the river Mukhrinskaya, Irtysh basin, West Siberia. Another morphologically and phenotypically similar methanotroph, strain OZ2, was isolated from a sediment of a subarctic freshwater lake, Archangel region, northern Russia. Cells of these three strains were Gram-stain-negative, light-pink-pigmented, non-motile, encapsulated, large cocci that contained an intracytoplasmic membrane system typical of type I methanotrophs. They possessed a particulate methane monooxygenase enzyme and utilized only methane and methanol. Strains Sph1T, Sph2 and OZ2 were able to grow at a pH range of 4.0–8.9 (optimum at pH 6.0–7.0) and at temperatures between 2 and 36 °C. Although their temperature optimum was at 20–25 °C, these methanotrophs grew well at lower temperatures, down to 4 °C. The major cellular fatty acids were C16:1ω5c, C16:1ω6c, C16:1ω7c, C16:1ω8c, C16:0 and C14:0; the DNA G+C content was 51.4–51.9 mol%. Strains Sph1T, Sph2 and OZ2 displayed nearly identical (99.1–99.7 % similarity) 16S rRNA gene sequences and belonged to the family *Methylococcaceae* of the class *Gammaproteobacteria*. The most closely related organism was *Methylovulum miyakonense* HT12T (96.0–96.5 % 16S rRNA gene sequence similarity and 90 % pmoA sequence similarity). The novel isolates, however, differed from *Methylovulum miyakonense* HT12T by cell morphology, pigmentation, absence of soluble methane monooxygenase, more active growth at low temperatures, growth over a broader pH range and higher DNA G+C content. On the basis of these differences, we propose a novel species, *Methylovulum psychrotolerans* sp. nov., to accommodate these methanotrophs. Strain Sph1T (=LMG 29227T=VKM B-3018T) is the type strain.

The genus *Methylovulum* belongs to the class *Gammaproteobacteria*, family *Methylococcaceae*, and is so far represented by the only species, *Methylovulum miyakonense*, which accommodates strictly aerobic, neutrophilic, obligate...
utilizers of C₃ compounds with type I intracytoplasmic membranes (ICM) and the ribulose-monophosphate pathway of carbon assimilation (Iguchi et al., 2011). The type strain of this species, Methylovulum miyakonense HT12ᵀ, was isolated from a forest soil and was characterized as a mesophilic bacterium with a growth optimum at 24–32°C. Several recent cultivation-independent studies, however, suggested that members of the genus Methylovulum are numerically abundant and metabolically active in low-temperature environments. Indeed, Methylovulum-like 16S rRNA gene sequences were detected using a stable isotope probing technique in sediments from an arctic lake in northern Alaska (He et al., 2012). These methanotrophs were also found in water discharged during summer seasons from Russell Glacier, a land-terminating outlet glacier at the western margin of the Greenland Ice Sheet (Dieser et al., 2014). In our recent study of cold methane seeps in floodplains of West Siberian rivers, Methylovulum-related bacteria were also identified among the dominant methanotroph groups (Oshkin et al., 2014). Our further efforts, therefore, were focused on obtaining cold-tolerant representatives of this genus in pure culture. Three strains of Methylovulum-like methanotrophs were obtained from two different permanently cold environments, i.e. West Siberian methane seeps and sediments of a subarctic freshwater lake. Here, we characterized these isolates and propose to classify them as belonging to a novel species of the genus Methylovulum.

Strains Sph1ᵀ and Sph2 were isolated from mud suspensions sampled from two methane seeps located at a distance of 300 m from each other in the valley of the river Mukhrinskaya, Irtysh basin, West Siberia (60° 53.358′ N 68° 42.486′ E). The seeps were characterized by low in situ temperatures (3.5 to 5°C), high concentrations of emitted methane (70–99% of gases released from these bubbling pools) and near-neutral pH of 6.8 to 6.9 (Oshkin et al., 2014). Aliquots (0.5 ml) of mud suspensions were placed in 120 ml serum bottles containing 20 ml of liquid diluted nitrate mineral salts medium (DNMS; Dunfield et al., 2003) with the addition of 0.1% (by volume) of a trace elements stock solution containing (in grams per litre) EDTA, 5; FeSO₄·7H₂O, 2; ZnSO₄·7H₂O, 0.1; MnCl₂·4H₂O, 0.03; CoCl₂·6H₂O, 0.2; CuCl₂·5H₂O, 0.1; NiCl₂·6H₂O, 0.02; and Na₂MoO₄·0.03. The medium pH was 6.8. The bottles were sealed with rubber septa, and CH₄ (30%, v/v) was added to the headspace using syringes equipped with disposable filters (0.22 µm). Bottles were incubated in static conditions at 9°C for 4 weeks until visible medium turbidity due to development of methanotrophic bacteria was observed. One of the major cell morphotypes in the resulting enrichment cultures was represented by large coccis, which could easily be recognized and traced in cultures by microscopic analysis. These cells became the main target of our further isolation efforts, which started with successive re-streaking of cell material from enrichment cultures on agar DNMS medium. Since development of large coccis was observed within a wide temperature range, the plates were further incubated at 20°C in desiccators under a methane-air (30:70) gas mixture. Colonies that appeared on the plates were picked randomly and examined microscopically in order to select for the target cell morphotype. Colonies composed mainly of large coccis were picked and transferred to the liquid medium MG2 with low salt content (in grams per litre) KH₂PO₄, 15; KNO₃, 15; MgSO₄, 15; NaCl, 20; CaCl₂·2H₂O, 10; trace elements 0.1% (v/v). Multiple dilution series in this medium with CH₄ (30%, v/v) as the growth substrate resulted in isolation of the target methanotrophic bacteria represented by large coccis (Fig. 1a), strains Sph1ᵀ and Sph2, as pure cultures.

Another morphologically similar isolate, designated strain OZ2, was obtained from sediments of a subarctic, shallow (1.5–2 m depth), unnamed freshwater lake, Archangelsk region, Northern Russia (67° 36.567′ N 33° 35.317′ E) using the same approach. This sampling site was also characterized by low in situ temperatures (5–7°C), and the sediment had a pH of 6.5. The sample was collected from the surface layer (0–3 cm) of sediments in the littoral zone of this lake (depth 0.4 m at the sampling site).

In order to identify strains Sph1ᵀ, Sph2 and OZ2 and to verify their relatedness, the 16S rRNA gene sequences of these bacteria
were determined. PCR-mediated amplification of the 16S rRNA gene was performed using primers 9f (5'-GAGTTTGATCMTGGCTCAG-3') and 1492r (5'-ACGGYTACCTTGT-TACGACTT-3') and reaction conditions described by Weisburg et al. (1991). Phylogenetic analysis was carried out using the ARB program package (Ludwig et al., 2004). The trees were reconstructed using distance-based (neighbour-joining), maximum-likelihood (DNAml) and maximum-parsimony methods. The significance levels of interior branch points obtained in neighbour-joining analysis were determined by bootstrap analysis (1000 data re-samplings) using PHYLIP (Felsenstein, 1989). The analysis revealed that strains SpH1, SpH2 and OZ2 possess nearly identical (99.1–99.7 % similarity) 16S rRNA gene similarity with Methylovulum miyakonense HT12, the neutrophilic and mesophilic methanotroph of the family Methylococcaceae, class Gammaproteobacteria (Fig. 2). Among taxonomically uncharacterized organisms, the highest 16S rRNA gene sequence similarity (99 % similarity) was observed with methanotrophic bacterium M200, which was isolated from a Sphagnum peat bog in the Netherlands (Kip et al., 2011). Since Methylovulum miyakonense was the closest taxonomically described relative of our isolates, the type strain of this species, DSM 23269T, was used as a reference organism in our study. It was maintained on NMS medium (Whittenbury et al., 1970; DSMZ medium no. 632), which was used for isolation and cultivation of Methylovulum miyakonense as described in the original publication (Iguchi et al., 2011). In all comparative tests, strains SpH1, SpH2, OZ2 and Methylovulum miyakonense DSM 23269T were also grown on NMS medium under identical growth conditions.

For growth in liquid media, 120 ml serum bottles were used with a headspace/liquid space ratio of 4:1. After inoculation, the bottles were sealed with silicone rubber septa, and methane was added aseptically using a syringe equipped with a disposable filter (0.22 µm) to achieve a 10–20 % mixing ratio in the headspace. Bottles were incubated on a rotary shaker (100 r.p.m.) at 20 °C. Culture purity was verified by examination under phase-contrast and electron microscopy and by plating on 10-fold diluted Luria–Bertani agar (1.0 % tryptone, 0.5 % yeast extract, 1.0 % NaCl). Only one cell morphotype was observed in cultures of strains SpH1, SpH2 and OZ2, and no growth on diluted Luria–Bertani agar was observed after 3 weeks of incubation.

Morphological observations and cell-size measurements were made with a Zeiss Axioplan 2 microscope and Axivision 4.2 software (Zeiss). Cells morphology was examined by using batch cultures grown to the early-exponential, late-exponential and stationary growth phases. Isolates SpH1, SpH2 and OZ2 were represented by Gram-stain-negative and non-motile cocci (3–5 µm in diameter), which reproduced by binary fission and occurred singly or in pairs (Fig. 1a). Morphologically, they were clearly different from

![Fig. 2. 16S rRNA gene based neighbour-joining tree showing the phylogenetic position of strains SpH1, SpH2 and OZ2 in relation to other members of the family Methylococcaceae. Bootstrap values (percentages of 1000 data resamplings) >80 % are shown. Black circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. The type II methanotrophs Methyloferula stellata AR4 (FR868343), Methylocella silvestris BL2 (AJ491847), Methylocapsa acidiphila B2 (AJ278726), Methylosinus sporium NCIMB 11126 (Y18946), Methylosinus trichosporium OB3b (Y18947) and Methylocystis parvus OB5 (Y18945) were used as an outgroup. Bar, 0.05 substitutions per nucleotide position.](http://ijs.microbiologyresearch.org)
cells of *Methylovulum miyakonense* DSM 23269<sup>T</sup> (Fig. 1b). Three-week-old colonies of strains Sph1<sup>T</sup>, Sph2 and OZ2 were round, 2–3 mm in diameter, slimy and light-pink with an entire edge and a smooth surface. Liquid cultures displayed white to pale-pink turbidity. Formation of a surface pellicle in static liquid cultures was not observed.

For preparation of ultrathin sections, cells of the exponentially growing culture of strain Sph1<sup>T</sup> were collected by centrifugation and pre-fixed with 1.5 % (w/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 6.5) for 1 h at 4 °C and then fixed with 1 % (w/v) OsO<sub>4</sub> in the same buffer for 4 h at 20 °C. After dehydration in an ethanol series, the samples were embedded into Epon 812 (Sigma-Adrich, USA) epoxy resin. Thin sections were cut on an LKB-4800 microtome (LKB-Produkter AB, Sweden), stained with 3 % (w/v) uranyl acetate in 70 % (v/v) ethanol, and then stained with lead citrate (Reynolds, 1963) at 20 °C for 4–5 min. The specimen samples were examined with a JEM-100B transmission electron microscope (JEOL, Japan) at an accelerating voltage of 80 kV. Examination of thin-sectioned cells of strain Sph1<sup>T</sup> revealed a typical Gram-negative structure of the cell wall and the presence of ICMs, arranged as stacks of vesicular discs (Fig. 1c), which is characteristic of type I methanotrophs.

Physiological tests were performed in liquid NMS medium with methane. Growth of strains Sph1<sup>T</sup>, Sph2 and OZ2 was observed in the presence of methane.

### Table 1. Phospholipid-derived fatty acid contents of strains Sph1<sup>T</sup>, Sph2 and OZ2 in comparison with *Methylovulum miyakonense* DSM 23269<sup>T</sup>

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>C&lt;sub&gt;14:1ω7c&lt;/sub&gt;</td>
<td>0.7</td>
<td>0.8</td>
<td>1.2</td>
<td>–</td>
</tr>
<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>9.3</td>
<td>7.1</td>
<td>9.2</td>
<td>7.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1ω8c&lt;/sub&gt;</td>
<td>25.3</td>
<td>30.1</td>
<td>22.7</td>
<td>33.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1ω7c&lt;/sub&gt;</td>
<td>28.7</td>
<td>22.5</td>
<td>33.0</td>
<td>36.1</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1ω6c&lt;/sub&gt;</td>
<td>6.2</td>
<td>6.4</td>
<td>5.7</td>
<td>6.1</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1ω5c&lt;/sub&gt;</td>
<td>17.9</td>
<td>17.3</td>
<td>19.2</td>
<td>15.3</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>6.3</td>
<td>11.4</td>
<td>6.2</td>
<td>12.1</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1ω9c&lt;/sub&gt;</td>
<td>–</td>
<td>1.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>βOH-nC&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>4.6</td>
<td>3.2</td>
<td>2.8</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**Fig. 3.** Unrooted neighbour-joining tree reconstructed on the basis of 141 deduced amino acid sites of partial *pmoA* gene sequences, showing the position of strains Sph1<sup>T</sup>, Sph2 and OZ2 relative to other type I and type II methanotrophs. Bootstrap values (percentages of 1000 data resamplings) >80 % are shown. Black circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 0.1 substitutions per amino acid position.
was monitored by measuring OD\textsubscript{600} for 2 weeks under a variety of conditions, including temperatures of 2–37 °C, pH 3.0–9.5 and NaCl concentrations of 0–5.0 % (w/v). Variations in the pH were achieved by mixing 0.1 M solutions of H\textsubscript{2}PO\textsubscript{4}, KH\textsubscript{2}PO\textsubscript{4}, K\textsubscript{2}HPO\textsubscript{4} and K\textsubscript{3}PO\textsubscript{4}. The utilization of potential carbon sources was examined using 0.1 % (w/v) concentrations of the following compounds: methylamine, formate, glucose, sucrose, galactose, lactose, fructose, citrate, succinate, pyruvate, acetate and tryptophane. The ability to grow on methanol was tested in NMS medium containing 0.01–6 % (v/v) methanol. The growth factor requirement was tested by supplementing NMS medium with 0.01 % (w/v) Bacto tryptone or 0.001 % (w/v) cyanocobalamin. Nitrogen sources were tested by replacing KNO\textsubscript{3} in liquid NMS medium with the following compounds at 0.05 % (w/v): ammonium chloride, sodium nitrate, urea, peptone, tryptone, yeast extract, Casamino acids, glycine, alanine, lysine, arginine, glutamate, glutamine, asparagine, tryptophan, methionine, threonine and histidine. For N\textsubscript{2}-fixation experiments, a nitrate-free NMS medium was used. Growth was examined after 1 month of incubation.

Strains Sph\textsuperscript{1T}, Sph\textsuperscript{2} and OZ2 were able to grow only on methane and methanol. The specific growth rate on methane under optimal growth conditions was 0.08–0.09 h\textsuperscript{−1} for strain Sph\textsuperscript{1} and 0.03–0.05 h\textsuperscript{−1} for strains Sph\textsuperscript{2} and OZ2. Growth factors were not required and also did not stimulate growth. By contrast, Methylovulum miyakonense DSM 23269\textsuperscript{1} grew better in the presence of growth factors, while it was only capable of growth in NMS medium without growth factors. Methanol supported growth of strains Sph\textsuperscript{1T}, Sph\textsuperscript{2} and OZ2 in the range of 0.1–5 % (v/v); the best growth occurred with 0.7 % (v/v). The specific growth rate on methanol was 0.024 h\textsuperscript{−1}. No growth was observed on multicharacter compounds. Nitrate, ammonium salts and Casamino acids were used as sources of nitrogen. The novel isolates were also capable of slow growth (OD\textsubscript{600} 0.15–0.20 after 3 weeks of incubation) in nitrogen-free medium under microoxic conditions (sealed flasks filled with liquid medium to 1/2 volume and with 30 % air, 20 % methane and 50 % nitrogen in the headspace). The njH (dinitrogen reductase) gene, however, could not be detected in our isolates using the primers described by Poly et al. (2001), although a PCR product of correct size was obtained in a positive control with DNA of Methylovulum miyakonense DSM 23269\textsuperscript{1}.

Strains Sph\textsuperscript{1T}, Sph\textsuperscript{2} and OZ2 grew in the pH range of 4.0–8.9, with an optimum at pH 6.0–7.0 (Fig. S1, available in the online Supplementary Material). The temperature range for growth was 2–32 °C for strains Sph\textsuperscript{1T} and OZ2 (Fig. S2) and 2–36 °C for strain Sph\textsuperscript{2}. Although their optimum temperature for growth was at 20–25 °C, our isolates grew very well at lower temperatures, down to 4 °C. Notably, the growth yield was always higher at 10 °C (OD\textsubscript{600} 1.8–2.0) than at 20 °C (OD\textsubscript{600} 1.2–1.5). As revealed in comparative tests, Methylovulum miyakonense DSM 23269\textsuperscript{1} was also able to grow at low temperatures, but its growth was less active than that of our isolates (Fig. S3). Freshwater isolates Sph\textsuperscript{1T}, Sph\textsuperscript{2} and OZ2 were highly sensitive to salt stress; their growth was inhibited at NaCl concentrations above 0.1 % (w/v).

For lipid analyses, strains Sph\textsuperscript{1T}, Sph\textsuperscript{2}, OZ2 and Methylovulum miyakonense DSM 23269\textsuperscript{1} were grown in parallel, at 20 °C, in liquid NMS medium with methane and harvested in the late exponential growth phase. Lipids were analysed following the procedure described by Simininge Damsté et al. (2011). The fatty acid profiles obtained for cells of strains Sph\textsuperscript{1T}, Sph\textsuperscript{2} and OZ2 were highly similar to each other (Table 1) and were defined by the predominance of monounsaturated C\textsubscript{16} fatty acids, which is typical for type I methanotrophs (Bowman et al., 1991, 1993). The major fatty acids were C\textsubscript{16:1}ω6c, C\textsubscript{16:0}, C\textsubscript{18:0}, C\textsubscript{18:1}ω9c, C\textsubscript{18:2}ω6c, C\textsubscript{16:0} and C\textsubscript{14:0} fatty acids. A highly similar fatty acid composition was previously reported for the closely related but taxonomically uncharacterized methanotroph, strain M200 (Kip et al., 2011). The double bond positions were determined by interpretation of the mass spectral fragmentation pattern of the DMDS (dimethyl disulfide) derivatives of the unsaturated fatty acids as described by Nicols et al. (1986). Notably, the fatty acid profile of Methylovulum miyakonense DSM 23269\textsuperscript{1} was similar to those of our isolates (Table 1). This was an unexpected finding because the original description of this species stated the absence of monounsaturated C\textsubscript{16} fatty acids and listed C\textsubscript{16:0} and C\textsubscript{14:0} as two major cellular fatty acids in Methylovulum miyakonense (Iguchi et al., 2011). In order to verify our data, we determined the partial (~700 bp) 16S rRNA gene sequence of the strain obtained from DSMZ and confirmed its identity with the respective gene sequence of Methylovulum miyakonense HT12\textsuperscript{4} deposited in GenBank under accession number AB501287. We then repeated cultivation, collected another batch of biomass of Methylovulum miyakonense

### Table 2. Major characteristics that distinguish Methylovulum psychrotolerans sp. nov. from Methylovulum miyakonense

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Cell shape</td>
<td>Cocc</td>
<td>Coccoid or short rods</td>
</tr>
<tr>
<td>Cell size (µm)</td>
<td>3–5</td>
<td>1.5–2.5×1.0–2.0</td>
</tr>
<tr>
<td>Colour of colonies</td>
<td>Light pink</td>
<td>Pale brown</td>
</tr>
<tr>
<td>Presence of sMMO</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>2–36</td>
<td>5–34</td>
</tr>
<tr>
<td>(optimum)</td>
<td>(20–25)</td>
<td>(24–32)</td>
</tr>
<tr>
<td>pH range</td>
<td>4.0–8.9</td>
<td>6.0–7.5</td>
</tr>
<tr>
<td>(pH optimum)</td>
<td>(6.0–7.0)</td>
<td>(6.5)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>51.3–51.9</td>
<td>50.7*</td>
</tr>
</tbody>
</table>

*Data are based on genome analysis (Hamilton et al., 2015).

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DSM 23269\textsuperscript{T} and repeated the fatty acid analysis. The latter confirmed the data shown in Table 1.

The DNA base composition of strains Sph\textsuperscript{1}, Sph2 and OZ2 was determined by thermal denaturation using a Unicam SP1800 spectrophotometer at a heating rate of 0.5 °C min\textsuperscript{-1}. The DNA G+C value (mol\%) was calculated according to the method of Owen \textit{et al.} (1969). The DNA of \\textit{Escherichia coli} K-12 was used as the standard. The DNA G+C content of our isolates was in the range of 51.4–51.9 mol\%.

Partial fragments of the \textit{pmoA} gene, which encodes the active-site polypeptide of particulate methane monooxygenase (pMMO), were amplified using the primers and reaction conditions described by Holmes \textit{et al.} (1995). Phylogenetic analysis based on fragments of the \textit{pmoA} gene revealed that strains Sph\textsuperscript{1T}, Sph2 and OZ2 display 90 % nucleotide sequence similarity (96.3 % derived amino acid sequence identity) to \textit{pmoA} gene fragments from \textit{Methylovulum miyakonense} HT12\textsuperscript{T} (Fig. 3). The \textit{mmoX} gene encoding a subunit of soluble methane monooxygenase (sMMO) could not be amplified from DNA of our isolates with any of the previously described \textit{mmoX}-targeted primers (Auman \textit{et al.}, 2000; McDonald \textit{et al.}, 2001; Miguez \textit{et al.}, 1997; Hutchens \textit{et al.}, 2004). The colorimetric naphthalene oxidation test (Graham \textit{et al.}, 1992) for sMMO activity in cells of strains Sph\textsuperscript{1}, Sph2 and OZ2 grown on Cu-free NMS medium was also negative, although bright purple colour developed on plates with sMMO-possessing \textit{Methylovulum miyakonense} DSM 23269\textsuperscript{T}, which was used as a positive control in this test. The results suggest that sMMO is not present in any of the three novel isolates.

In summary, 16S rRNA and \textit{pmoA} gene phylogenies as well as fatty acid profiles characterize strains Sph\textsuperscript{1T}, Sph2 and OZ2 as members of the genus \textit{Methylovulum}. However, our novel isolates differed from the only so far described species of this genus, \textit{Methylovulum miyakonense}, by cell morphology, pigmentation, absence of sMMO, more active growth at low temperatures, broader pH range for growth and higher DNA G+C content (Table 2). Based on these differences, we propose to classify strains Sph\textsuperscript{1T}, Sph2 and OZ2 as belonging to a novel, cold-adapted species of the genus \textit{Methylovulum}, \textit{Methylovulum psychrotolerans} sp. nov.

**Emended description of the genus Methylovulum**

Cells are Gram-stain-negative, aerobic, non-motile, coccolid- or short-rod-shaped and possess stacks of ICMs, typical of type I methanotrophs. No cysts are formed. Growth is observed on methane and methanol as sole carbon sources. Methane is oxidized by pMMO; the presence of sMMO is variable. 
\textit{COTT} compounds are assimilated via the ribulose polyphosphate pathway. Mesophilic and psychrotolerant. Growth of some species may be stimulated by growth factors. Major cellular fatty acids are C\textsubscript{16:1ω5c}, C\textsubscript{16:1ω6c}, C\textsubscript{16:1ω7c}, C\textsubscript{16:1ω6c}, C\textsubscript{16:1ω7c}, C\textsubscript{16:1ω6c} and C\textsubscript{14:0}. The DNA G+C content is 50.7–51.9 mol\%. Phylogenetically, a member of the family \textit{Methylcocaceae}, in the class \textit{Gammaproteobacteria}. The type species is \textit{Methylovulum miyakonense}.

**Description of Methylovulum psychrotolerans** sp. nov.

\textit{Methylovulum psychrotolerans} (psy.chro.to’le.rans. Gr. adj. psychros cold; L. pres. part. tolerans tolerant; N.L. part. adj. psychrotolerans cold-tolerant).

Gram-stain-negative, non-motile cocci, 3–5 μm in diameter. Cells occur singly or in pairs and are covered by large capsules. Possess stacks of ICMs typical of type I methanotrophs. Colonies are slimy and lightpink with an entire edge and a smooth surface. Liquid cultures display white to pale-pink homogeneous turbidity; no surface pellicle is formed. The temperature range for growth is 2–36 °C with an optimum at 20–25 °C. Growth occurs between pH 4.0 and 8.9 with an optimum at pH 6.0–7.0. Methane and methanol are the only growth substrates. Methane is oxidized by pMMO; sMMO is absent. Methanol is utilized at concentrations 0.1–5.0 % (v/v); optimal growth occurs with 0.7 % (v/v) CH\textsubscript{3}OH. Growth factors are not required. NaCl inhibits growth at concentrations above 0.1 %. The predominant fatty acids are C\textsubscript{16:1ω5c}, C\textsubscript{16:1ω6c}, C\textsubscript{16:1ω8c}, C\textsubscript{16:1ω7c}, C\textsubscript{16:0} and C\textsubscript{14:0}. The DNA G+C content is 51.4–51.9 mol\%.

The type strain, Sph\textsuperscript{1T} (=LMG 29227\textsuperscript{T}=VKM B-3018\textsuperscript{T}), was isolated from a cold methane seep in West Siberia.

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