Methyloparacoccus murrellii gen. nov., sp. nov., a methanotroph isolated from pond water

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Two novel methanotrophic strains, R-49797T and OS501, were isolated from pond water in South Africa and Japan, respectively. Strains R-49797T and OS501 shared 99.7 % 16S rRNA gene sequence similarity. Cells were Gram-stain-negative, non-motile cocci with a diplococcoid tendency and contained type I methanotroph intracytoplasmic membranes. The pmoA gene encoding particulate methane monooxygenase was present. Soluble methane monooxygenase (sMMO) activity, the mmoX gene encoding sMMO and the nifH gene encoding nitrogenase were not detected. Methane and methanol were utilized as sole carbon source. The strains grew optimally at 25–33 °C (range 20–37 °C) and at pH 6.3–6.8 (range 5.8–9.0). The strains did not support growth in media supplemented with 1 % (w/v) NaCl. For both strains, the two major fatty acids were C16:1ω7c and C16:0 and the DNA G+C content was 65.6 mol%. The isolates belong to the family Methylococcaceae of the class Gammaproteobacteria and cluster most closely among the genera Methylocaldum, Methylococcus and Methylogaea, with a 16S rRNA gene sequence similarity of 94.2 % between strain R-49797T and its closest related type strain (Methylocaldum gracile VKM 14LT). Based on the low 16S rRNA gene sequence similarities with its nearest phylogenetic neighbouring genera, the formation of a separate lineage based on 16S rRNA and pmoA gene phylogenetic analysis, and the unique combination of phenotypic characteristics of the two isolated strains compared with the genera Methylocaldum, Methylococcus and Methylogaea, we propose to classify these strains as representing a novel species of a new genus, Methyloparacoccus murrellii gen. nov., sp. nov., within the family Methylococcaceae. The type strain of Methyloparacoccus murrellii is R-49797T (=LMG 27482T =JCM 19379T).

Aerobic methanotrophic bacteria are defined by their ability to oxidize methane and utilize it as carbon and energy source using a methane monooxygenase (MMO), of which a soluble (sMMO) and a particulate (pMMO) form have been described (Semrau et al., 2010). These methanotrophs are ubiquitous in nature and have been found in different habitats where oxygen and methane are readily available, such as in soils, sediments, wetlands, freshwater and marine environments (Bowman, 2006; Hanson & Hanson, 1996). Although non-proteobacterial methanotrophs have been described (Ettwig et al., 2010; Op den Camp et al., 2009), the methanotrophs that belong to the classes Gammaproteobacteria or Alphaproteobacteria have been studied much more intensively (Bowman et al., 1993). Twelve methanotrophic genera have been formally named and described within the family Methylococcaceae of the class Gammaproteobacteria: the genera Methylococcus, Methylocaldum, Methylogaea, Methylothermus, Methylohalobius, Methylosoma, Methylovulum, Methylophaera, Methylobacter, Methylobacterium, Methylosarcina and Methylosomas.
(Geymonat et al., 2011). Within the class Alphaproteobacteria, the genera Methylosinus and Methylocystis in the family Methyloctaceae and the genera Methylocella, Methyloferula and Methylocapsa in the family Beijerinckiaceae have been named and described (Dedysh, 2009; Vorobev et al., 2011).

Recently, the genus Methylogaea was named through the formal characterization of a non-thermotolerant strain within the Methylococcus–Methylocaldum clade, which typically harbours thermotolerant members (Geymonat et al., 2011). Here, we report the characterization of two novel isolates within this Methylococcus–Methylocaldum–Methylogaea clade that (i) share similar cell-shape characteristics with Methylococcus strains, (ii) are genotypically related most closely to Methylocaldum strains and (iii) are non-thermotolerant, as with the type species of the genus Methylogaea. Furthermore, the nifH gene, which is commonly present in members of this clade, could not be detected for the two novel isolates.

Strain R-49797T was isolated from a facultative waste stabilization pond in South Africa (33° 19′ 07″ S 26° 33′ 25″ E) by miniaturized extinction culturing (Hoefman et al., 2012a) in diluted ammonium mineral salts (dAMS) medium, which was a modification of diluted nitrate mineral salts medium (dNMS; Hoefman, et al., 2012a) amended with 2 mM NH₄Cl as sole nitrogen source, 4 mM phosphate buffer and 10 μM CuSO₄. Strain OS501 was isolated from pond water from Inukai Pond in Suita City, Osaka, Japan (34° 49′ 09″ N 135° 31′ 42″ E), by plating of enrichment cultures (NMS medium, 20% CH₄ in air, 28 °C, shaking) on NMS agar (ATCC 1306 medium) and incubation in gas-tight jars under a CH₄/air atmosphere. Purity of the isolates was assessed as described by Hoefman et al. (2012a).

In the present study, both strains were routinely cultured at 28 °C in dNMS (liquid or solidified with agar) with a modified copper concentration (10 μM Cu²⁺) under a CH₄/air atmosphere in gas-tight flasks (20% methane in air) or jars (CH₄/air, 1:1).

Cell morphology and motility were assessed by phase-contrast microscopy. Gram staining was performed, and cytochrome c oxidase and catalase activities were assessed according to Cleenewerck et al. (2002). Flagella staining was performed according to Heimbrook et al. (1989). The presence of Azotobacter-type cysts was evaluated by the staining procedure of Vela & Wyss (1964) on cultures grown to stationary phase. Heat resistance and desiccation resistance were assessed on turbid suspensions (OD₆₀₀ of 1) of cultures grown to stationary phase as described by Whittenbury et al., (1970a). Transmission electron microscopy was performed according to Speck & Lipski (2011).

The ability to fix nitrogen was assessed at normal (21%) and tenfold reduced (2.1%) oxygen levels, as described previously (Hoefman et al., 2014). The ability to grow using the following carbon sources (0.1%, w/v) was evaluated in liquid dNMS without methane added to the headspace: methanol, formate, urea, methylamine, dimethylamine, dimethyl carbamate, formamide, acetate, pyruvate, succinate, malate, ethanol, citrate, glycine, D-xylose, maltose and glucose. Utilization of the following nitrogen sources at a concentration of 2 mM was evaluated in liquid dMS (dNMS without nitrogen source) with methane added to the headspace: nitrate, nitrite, ammonium [NH₄Cl and (NH₄)₂SO₄], formamide, methylamine, glycine, hydroxylamine, urea, L-serine, L-proline, L-aspartate, L-leucine, L-cysteine, L-arginine and yeast extract (0.1%, w/v, yeast extract, instead of 2 mM). Salt tolerance [tested at 0.23% (40 mM), 0.58% (100 mM), 1%, 5% and 10% NaCl spiked in dNMS], the pH optimum and range (tested at pH 4, 5, 5.8, 6.3, 6.8, 7.3, 7.8, 9 and 10) and the temperature optimum and range (tested at 5, 15, 20, 25, 28, 33, 37, 45 and 52 °C) of the strains were assessed in dNMS.

Nitrate reduction was evaluated by growth in liquid dNMS amended with 10 mM KNO₃ and monitoring of nitrite production by colorimetry (Griess, 1879) and N₂O production by gas chromatography (Compact GC; Global Analyser Solutions). sMMO activity was tested using a modified version of the naphthalene oxidation assay (Hoefman, et al., 2012a). Cryopreservation of strains R-49797T and OS501 with 5% DMSO as cryoprotectant at −80 °C and in liquid nitrogen was performed as described by Hoefman et al. (2012b).

DNA was extracted and 16S rRNA gene sequences were generated as described by Hoefman et al. (2012a). Sequences were assembled with BioNumerics 5.1 (Applied Maths). Preliminary genus identification was obtained by query in the ‘Classifier’ program of the Ribosomal Database Project II (Cole et al., 2005). Sequences of strains R-49797T and OS501 were aligned among a selection of methanotrophic type strains using the integrated aligner of ARB (Ludwig et al., 2004). Based on the 16S rRNA gene alignment, a maximum-likelihood (ML) phylogenetic tree was reconstructed using RAxML v7.3.5 based on the General Time Reversible substitution model with gamma-distributed rate variation (GTR + Gamma). Bootstrap analysis based on 1000 replicates was performed. The best scoring ML tree was exported in newick format and analysed using MEGA 5 software (Tamura et al., 2011).

Amplification of the pmoA gene encoding the 27 kDa peptide of PMMO was carried out with the A189f/m6661r primer set according to Costello & Lidstrom (1999). Amplification of the mmoX gene encoding the β-subunit of the hydroxylase component of sMMO was tested with primer sets 534f/1393r (Horz et al., 2001), mmoXA/mmoXB (Auman et al., 2000) and mmoX206f/mmoX886r (Hutchens et al., 2004). Amplification of the pzmA [encoding a sequence-divergent particulate monooxygenase (pXMO)], nifH (encoding the highly conserved Fe protein of nitrogenase) and nifAF (encoding the large subunit of methylno dehydrogenase) genes was performed using the primer sets pmoA189F/pzmA634R (Tavormina et al., 2008),
F1/nifH439R (De Meyer et al., 2011) and f1003/r1561 (McDonald et al., 1995), respectively. For detection of each of the above-mentioned genes, the PCR mix and temperature program of the original studies were applied; the outcome of each test was verified using a set of methanotrophic type strains as positive and negative controls.

**Fig. 1.** (a) Phase-contrast micrograph of actively growing cells of strain R-4979T\(^\text{T}\) showing cocci with a dipplococcoid tendency, similar to those reported for *Methylococcus capsulatus* (Foster & Davis, 1966). (b, c) Electron micrographs of ultrathin sections of actively growing cells of strain R-4979T\(^\text{T}\) showing a cell shape resembling *Methylococcus* cultures and displaying typical gammaproteobacterial methanotrophic intracytoplasmic membrane arrangements [ICM (b)]. Two types of unidentified storage compounds are observed [dark and light circles (b)]. Bars, 0.2 \(\mu\)m (b), 1 \(\mu\)m (c).

**Fig. 2.** 16S rRNA gene phylogenetic ML tree (1223 nt alignment; GTR+Gamma substitution model; 1000-replicate bootstrapping) of strains R-4979T\(^\text{T}\) and OS501 with the type strains of recognized species of the genera *Methylococcus*, *Methylocaldum* and *Methylogaea* and the type strains of the remaining genera within the family *Methylococcaceae*. The alphaproteobacterial methanotrophs *Methylocystis parvus* NCIMB 11129\(^\text{T}\) (GenBank accession number Y18945), *Methylosinus trichosporium* OB3b\(^\text{T}\) (Y18947), *Methyloferula stellata* AR4\(^\text{T}\) (FR686343), *Methylocella palustris* K\(^\text{T}\) (Y17144) and *Methylocapsa acidiphila* B2\(^\text{T}\) (AJ278726) were used as an outgroup. Bootstrap values below 70% are not shown. Bar, 0.01 substitutions per nucleotide position.
When functional gene amplification was positive, sequences were generated as explained for the 16S rRNA gene analysis and subsequently placed in the correct reading frame as determined by Transeq (www.ebi.ac.uk/tools/emboss/transeq) and protein translation analysis using pBLAST (Altschul et al., 1990). Sequences were aligned using MEGA 5 software based on translated protein sequences, and DNA-based ML phylogenetic trees were reconstructed using RAxML as explained above.

DNA was extracted and purified as described by Logan et al. (2000) to determine the G+C content by HPLC (Mesbah et al., 1989). Phospholipid fatty acid analysis was carried out as described by Vancanneyt et al. (1996). Fatty acids were identified using the Microbial Identification System (MIDI) software (Sherlock Microbial Identification Systems, version 3.0; Library TSBA 50, version 5.0).

One-week-old colonies of strains R-49797T and OS501 were white to creamy and round. Two-week-old colonies of strain OS501, but not of strain R-49797T, were still mostly white but displayed a distinct shiny green appearance. Cells of both strains were Gram-stain-negative, non-motile cocci (diameter 0.8–1.5 μm) that displayed a diplococcoid tendency (Fig. 1a), similar to what was observed by Foster & Davis (1966) for *Methylococcus capsulatus* cultures. Cells exhibited catalase and cytochrome c oxidase activities. Azotobacter-type cysts were not observed. Cells were neither heat-resistant nor desiccation-resistant. Transmission electron micrographs showed cells with intracytoplasmic membrane systems appearing as bundles of vesicular discs (Fig. 1b, c), a typical feature of gammaproteobacterial methanotrophs (Hanson & Hanson, 1996). Similarly to that observed for the type strain of *Methylogaea oryzae* (Geymonat, et al., 2011), cells of the novel strains stored two types of

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**Fig. 3.** *pmoA* gene phylogenetic ML tree (438 nt alignment; GTR+Gamma substitution model; 1000-replicate bootstrapping) of strains R-49797T and OS501 with the available *pmoA* gene sequences of type strains within the genera *Methylococcus*, *Methylocaldum* and *Methylogaea* and one *pmoA* gene sequence per remaining genus within the family *Methylococcaceae*. The alphaproteobacterial methanotrophs *Methylocystis parvus* OBBP T (GenBank accession number U31651), *Methylomus trichosporium* IMET 10561T (AJ459001) and *Methylocapsa acidiphila* B2T (AJ278727) were used as an outgroup. Bootstrap values below 70% are not shown. Bar, 0.05 substitutions per nucleotide position.
compounds displayed as light and dark entities (Fig. 1b). The inclusions of low electron density possibly represent poly-β-hydroxybutyrate granules, known to be produced by several methanotrophs (Helm et al., 2006; Heyer et al., 2005; Whittenbury et al., 1970b). Strains R-49797\(^T\) and OS501 could be cryopreserved successfully at \(-80^\circ\)C and in liquid nitrogen using 5 % DMSO as cryoprotectant.

Strains R-49797\(^T\) and OS501 utilized only methanol or methanol as sole carbon and energy source. Medium amended with 0.1 % methanol did not support growth, but the strains grew with methanol fumes as sole carbon source when incubated on dNMS plates with a few drops of methanol placed on the inside lid of the Petri dish. Although growth of strain OS501 could be improved by the addition of cobalamin during cultivation (Iguchi et al., 2011), this characteristic appeared to be strain-specific as such improved growth was not observed for strain R-49797\(^T\) with vitamin B12 at 0.1 μg l\(^{-1}\) added to the medium.

Growth was observed with ammonium [both NH\(_4\)Cl and (NH\(_4\))\(_2\)SO\(_4\)], nitrate, nitrite, urea, proline, aspartate, arginine and yeast extract as sole nitrogen source. The isolates could not grow in nitrogen-free medium at high (21 %) or low (2.1 %) oxygen tension and the pxmA gene could not be amplified. The strains grew at 20–37 °C (optimum 25–33 °C), at pH 5.8–9 (optimum 6.3–6.8) and with up to 100 mM NaCl. Under optimal conditions in dNMS, the doubling time of strains R49797\(^T\) and OS501 was 27–28 and 15–18 h, respectively. Nitrate reduction of both strains was confirmed by the formation of nitrite and N\(_2\)O following incubation with 10 mM KNO\(_3\) as sole nitrogen source. The strains were negative for sMMO activity and the mmoX gene could not be amplified. The pmoA gene was present, while the pxmA gene could not be detected by PCR.

16S rRNA gene sequences of strains R-49797\(^T\) (1451 bp) and OS501 (1497 bp) were obtained. Phylogenetic analysis showed that the two strains formed a new cluster within the Methylococcus–Methylocaldum–Methylogaea clade (Fig. 2). Pairwise 16S rRNA gene sequence comparisons showed that strain R-49797\(^T\) shared 93.8–94.2 % similarity with the type strains of the three recognized species of the genus Methylocaldum (strains VKM 14L\(^T\), OR2\(^T\) and LK6\(^T\)), 93.8 % with Methylococcus capsulatus Texas\(^T\), 90.0 % with Methylogaea oryzae E10\(^T\) and 99.7 % with strain OS501. The 16S rRNA gene sequence results were supported by phylogenetic analysis and pairwise comparison of the pmoA gene, which confirmed that strains R-49797\(^T\) and OS501 form a line of descent different from those of the other members of the Methylococcus–Methylocaldum–Methylogaea clade (Fig. 3). Pairwise pmoA gene sequence comparison showed that strain R-49797\(^T\) shared 99.8 % similarity with strain OS501, 86.0 % with Methylococcus capsulatus Bath, 84.0 % with Methylogaea oryzae E10\(^T\), 84.7 % with Methylocaldum gracile VKM 14L\(^T\), 82.1 % with Methylocaldum tepidum LK6\(^T\) and 81.3 % with Methylocaldum szegediense OR2\(^T\).

The DNA G+C content as determined by HPLC was 65.6 mol% for strains R-49797\(^T\) and OS501. Similar values are observed for other members of the Methylococcus–Methylocaldum–Methylogaea clade. Phospholipid fatty acid analysis identified C\(_{16:1}\)\(\delta^7\)c and C\(_{16:0}\) as the two major fatty acids. This finding is consistent with the other members of the Methylococcus–Methylocaldum–Methylogaea clade, although the most dominant fatty acid of strains R-49797\(^T\) and OS501 was C\(_{16:1}\)\(\delta^7\)c while for the other members of the clade C\(_{16:0}\) is more dominant than C\(_{16:1}\)\(\delta^7\)c (Table 1).

The major morphological and physiological characteristics distinguishing strains R-49797\(^T\) and OS501 from other genera within the Methylococcus–Methylocaldum–Methylogaea clade are presented in Table 2. Cells of strains R-49797\(^T\) and OS501 are clearly different in appearance when compared with those of members of the genera Methylogaea and Methylocaldum, as rod-shapes, pleomorphism and motility were absent for strains R-49797\(^T\) and OS501. The non-motile cocci most closely resemble the appearance of cells of Methylococcus strains. Although strains representing the genera Methylococcus and Methylocaldum are thermotolerant, strains R-49797\(^T\), OS501 and Methylogaea oryzae E10\(^T\) are not. Further distinctions between strains R-49797\(^T\) and OS501 and Methylococcus strains include sensitivity to 1 % NaCl and sensitivity to 0.1 % methanol and absence of the pxnH and mmoX genes.

**Table 1.** Composition of cellular fatty acids distinguishing strains R-49797\(^T\) and OS501 from other genera within the Methylococcus–Methylocaldum–Methylogaea clade

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_{12}:0)</td>
<td>–</td>
<td>–</td>
<td>NR</td>
<td>2.1</td>
<td>0–0.1</td>
</tr>
<tr>
<td>C(_{14}:0)</td>
<td>4.7</td>
<td>3.8</td>
<td>0.8–6.2</td>
<td>5.8</td>
<td>2.0–2.4</td>
</tr>
<tr>
<td>C(_{15:08\delta^7)c</td>
<td>0.3</td>
<td>3</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>C(_{16}:0)</td>
<td>3.2</td>
<td>3.3</td>
<td>0–12.7</td>
<td>1.0</td>
<td>2.5–3.5</td>
</tr>
<tr>
<td>C(_{16}:1)(\delta^9)c</td>
<td>6.5</td>
<td>5.1</td>
<td>–</td>
<td>7.4</td>
<td>NR</td>
</tr>
<tr>
<td>C(_{16}:1)(\delta^7)c</td>
<td>54.2</td>
<td>52.4</td>
<td>10.6–45.9</td>
<td>10.3</td>
<td>NR</td>
</tr>
<tr>
<td>C(_{16}:1)(\delta^5)c</td>
<td>4.2</td>
<td>5.7</td>
<td>0–9.0</td>
<td>NR</td>
<td>–</td>
</tr>
<tr>
<td>C(_{16}:1)(\delta^1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11.9–13.3</td>
</tr>
<tr>
<td>C(_{16}:0)</td>
<td>23.7</td>
<td>24.8</td>
<td>33.5–56</td>
<td>62.1</td>
<td>63.7–65</td>
</tr>
<tr>
<td>iso-C(_{16}:0) 3-OH</td>
<td>2.6</td>
<td>2.7</td>
<td>NR</td>
<td>2.9</td>
<td>–</td>
</tr>
<tr>
<td>C(_{16}:0) 3-OH</td>
<td>–</td>
<td>–</td>
<td>NR</td>
<td>3.7</td>
<td>NR</td>
</tr>
<tr>
<td>C(_{17}:0) cyclo</td>
<td>–</td>
<td>–</td>
<td>0–15.1</td>
<td>–</td>
<td>6.1–9.00</td>
</tr>
</tbody>
</table>

*This peak in the chromatogram represents C\(_{16}:1\)\(\delta^7\)c and/or iso-C\(_{15}:0\) 2-OH according to the MIDI system; however, in this study the peak is assigned to C\(_{16}:1\)\(\delta^7\)c as this fatty acid is common among the studied methanotrophs.
Table 2. Major characteristics distinguishing strains R-49797T and OS501 from other genera within the Methylocaldum–Methylocaldum–Methylogae clade

Taxa: 1, strains R-49797T and OS501; 2, Methylococcus (data from Bowman et al., 1993); 3, Methylogae oryzae (Geymonat et al., 2011); 4, Methylocaldum (Bodrossy et al., 1997; Eshinimaev et al., 2004). +, Positive result; −, negative result; NR, not reported.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Cocci</td>
<td>Cocci–rods</td>
<td>Curved rods</td>
<td>Rods–pleomorphic</td>
</tr>
<tr>
<td>Cell size (μm)</td>
<td>0.8–1.5</td>
<td>0.8–1.5 × 1.0–1.5</td>
<td>0.5–0.7 × 2.0–2.2</td>
<td>0.4–1.2 × 1.0–2.0</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>White</td>
<td>White to brown</td>
<td>White</td>
<td>Cream to brown</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>Variable</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Chain formation</td>
<td>–</td>
<td>–</td>
<td>NR</td>
<td>Variable</td>
</tr>
<tr>
<td>Cyst formation</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>20–37</td>
<td>28–55</td>
<td>20–37</td>
<td>20–62</td>
</tr>
<tr>
<td>Temperature optimum (°C)</td>
<td>25–33</td>
<td>37–50</td>
<td>30–35</td>
<td>42–55</td>
</tr>
<tr>
<td>pH range</td>
<td>5.8–9</td>
<td>5.5–9.0</td>
<td>5–8</td>
<td>6–8.5</td>
</tr>
<tr>
<td>pH optimum</td>
<td>6.3–6.8</td>
<td>NR</td>
<td>6.5–6.8</td>
<td>7.1–7.2</td>
</tr>
<tr>
<td>Tolerance to 1 % NaCl</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Presence of nifH gene</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>N2 fixation</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>NR</td>
</tr>
<tr>
<td>sMMO</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth with 0.1 % methanol</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>65.6</td>
<td>59–66</td>
<td>63.1</td>
<td>57–59</td>
</tr>
</tbody>
</table>

*Data on the presence of the nifH gene for the type strains of species of the genus Methylocaldum have not been reported, but nifH gene sequences of strains reported by Eshinimaev et al. (2004) are available.

resulting in the absence of nitrogenase and sMMO activity, respectively, for strains R-49797T and OS501.

Given the above-mentioned genotypic and phenotypic differences between strains R-49797T and OS501 and other members of the Methylococcus–Methylocaldum–Methylogae clade, we propose to assign them to a novel species of a new genus, Methyloparacoccus murrelli gen. nov., sp. nov.

Description of Methyloparacoccus gen. nov.

Methyloparacoccus [Me.thy.lo.pa.ra.cocc.us. N.L. n. methyllum (from French méthyle) the methyl group; N.L. pref. methylo- pertaining to the methyl radical; Gr. prep. para beside, alongside of, near, like; N.L. masc. n. coccus (from Gr. n. kokkos) a grain or berry; N.L. masc. n. Methyloparacoccus referring to a methyl-using organism resembling but clearly different from other methyl-using cocci].

Cells are aerobic, Gram-stain-negative, non-motile, coccoid and show a diplococcioid tendency. Cells possess the typical intracytoplasmic membrane system for gammaproteobacterial methanotrophs forming bundles of membrane vesicles. Resting stages are not observed. Cells utilize only methane or methanol as sole carbon source. Methane is oxidized by pMMO; sMMO and pXMO are not present. N2 is not fixed, and the nifH gene is absent. Cells are neutrophilic, mesophilic and non-thermotolerant. The most dominant cellular fatty acids are C16:1ω7c and C16:0. The most closely related genera are Methylocaldum, Methylococcus and Methylogae within the family Methylococcaceae in the class Gammaproteobacteria. The type species is Methyloparacoccus murrelli.

Description of Methyloparacoccus murrelli sp. nov. Methyloparacoccus murrelli (mur.rell’i.i. murrelli of Murrell, named in honour of the British microbiologist Colin Murrell for his contributions to the knowledge on methanotrophs).

Displays the properties as described for the genus. In addition, cocci have a diameter of 0.8–1.5 μm. Cells grow optimally at 25 °C–33 °C and at pH 6.3–6.8 and can utilize ammonium [both NH4Cl and (NH4)2SO4], nitrate, nitrite, urea, proline, aspartate, arginine and yeast extract as sole nitrogen source. Cells grow with 0.58 % NaCl amended to the medium, but are sensitive to addition of 1 % NaCl. The type strain, R-49797T (=LMG 27482T =JCM 19379T), was isolated from pond water in South Africa. The G+C content of the type strain is 65.6 mol%. Strain OS501 (=LMG 27483) was isolated from pond water in Japan and is a second strain of the species.

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