Hyphomicrobium chloromethanicum sp. nov. and Methylobacterium chloromethanicum sp. nov., chloromethane-utilizing bacteria isolated from a polluted environment

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Two chloromethane-utilizing facultatively methylotrophic bacteria, strains CM2T and CM4T, were isolated from soil at a petrochemical factory. On the basis of their morphological, physiological and genotypical properties, strain CM2T (= VKM B-2176T = NCIMB 13687T) is proposed as a new species of the genus Hyphomicrobium, Hyphomicrobium chloromethanicum, and strain CM4T (= VKM B-2223T = NCIMB 13688T) as a new species of the genus Methylobacterium, Methylobacterium chloromethanicum.

Keywords: chloromethane, Hyphomicrobium, Methylobacterium

Chloromethane (methyl chloride; CH₃Cl) is the most abundant halocarbon in the atmosphere and is responsible for 15–20% of chlorine-catalysed ozone destruction in the stratosphere. It is released at an estimated global rate of 3–5 × 10⁶ tons per year, primarily from natural sources, with less than 1% of the flux from industrial sources (Khalil et al., 1999). Eight strains of chloromethane-utilizing bacteria (CM1, CM2, CM4, CM9, CM29, CM30, CM34 and CM35) were isolated from soil samples near a petrochemical factory in Russia (Doronina et al., 1996). The phenotypic characteristics of these strains indicated that they were of the genera Hyphomicrobium (CM1, CM2, CM9, CM29, CM30, CM34 and CM35) were isolated from soil samples near a petrochemical factory in Russia (Doronina et al., 1996). The phenotypic characteristics of these strains indicated that they were of the genera Hyphomicrobium (CM1, CM2, CM9, CM29, CM30, CM34 and CM35) were isolated from soil samples near a petrochemical factory in Russia (Doronina et al., 1996). The Hyphomicrobium strains had characteristics typical of the type species Hyphomicrobium vulgaris except for their growth on chloromethane. The cells were rod-shaped, 0.3–1.2 × 1–3 μm, having polar prosthecae. The bacteria were appendaged, reproduced by budding (Hirsch, 1989), and were restricted facultative methylotrophs capable of growth on one-carbon compounds such as methanol and methylamine (Harder & Attwood, 1978).

The Methylobacterium strains (CM4, CM30, CM34) have characteristics typical of the type species Methylobacterium organophilum apart from their growth on chloromethane. Cells were rod-shaped, 0.8–1.0 × 1.0–8.0 μm, occurring singly or occasionally in rosettes. Motility occurs by a single flagellum, cells stain Gram-negative and colonies are pigmented pale pink on methanol salts agar plates. They are strictly aerobic, catalase- and oxidase-positive, and the optimum temperature for growth is 25–30 °C (Green & Bousfield, 1983; Green et al., 1988). Methylobacterium sp. strain CM4T has been studied to determine a pathway for conversion of chloromethane to formate (Vannelli et al., 1998, 1999; Studer et al., 1999). It was shown that the protein CmuA first transfers the methyl group of chloromethane to a corrinoid protein, from where it is transferred to tetrahydrofolate by the protein CmuB. Both CmuA and CmuB displayed sequence similarity to methyltransferases of methanogenic members of the domain Archaea. As well as growing on chloromethane, CM2T and CM4T will also oxidize, but not grow on, bromomethane and iodomethane (C. McAnulla and others, unpublished; Vannelli et al., 1998). Here we report the phylogenetic characterization based on the 16S rRNA gene sequences, and propose names for strains CM2T and CM4T.

The DNA of strains CM2T, CM4T, CM29 and CM30 was extracted (Marmur, 1961), and the 16S rRNA genes were amplified and sequenced (Lane, 1991). Complete 16S rRNA sequences were obtained for strains CM2T and CM4T, and partial sequences (700 bp) were obtained for strains CM29 and CM30. The four sequences were compared using the Genetics

The GenBank accession numbers for the 16S rRNA gene sequence of strains CM2T and CM4T are AF198623 and AF198624, respectively.
Fig. 1. Phylogenetic analysis of the 16S rRNA sequences of Hyphomicrobium sp. strain CM2¹ and other Hyphomicrobium strains and related genera. The dendrogram shows the results of an analysis in which DNADIST was used. Bootstrap values greater than 50% derived from 100 replicates are also shown. The bar represents 10% sequence divergence, as determined by measuring the lengths of the horizontal lines connecting any two species.

Fig. 2. Phylogenetic analysis of the 16S rRNA sequences of Methylobacterium sp. strain CM4¹ and other Methylobacterium strains and related genera. The dendrogram shows the results of an analysis in which DNADIST was used. Bootstrap values greater than 50% derived from 100 replicates are also shown. The bar represents 10% sequence divergence, as determined by measuring the lengths of the horizontal lines connecting any two species.

Computer Group Sequence analysis software package version 8.0 (Genetics Computer Group, Madison, Wisconsin). Hyphomicrobium strains CM2¹ and CM29 were found to be identical, and Methylobacterium strains CM4¹ and CM30 were also identical to each other. The complete 16S rRNA gene sequences from strains CM2¹ and CM4¹ were aligned, using the ARB program for sequence alignment (O. Strunk and others; http://www.mikro.biologie.tu-muenchen.de), to representative organisms from the same and related genera of bacteria, and their phylogenetic position was determined using the DNADIST, DNAML, D napARS and SEQBOOT programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic dendrograms were constructed from the distance data using the Fitch-Margoliash method and the dendrograms were drawn using the TreeView program version 1.5 (Page, 1996). Phylogenetic analysis of the 16S rDNA of strain CM2¹ (Fig. 1) showed it to be located within cluster II of the Hyphomicrobium species (Rainey et al., 1998), as supported by bootstrap values. The 16S rDNA of strain CM2¹ has high sequence similarity with Hyphomicrobium facile subsp. facile H-526¹ (97.1%), Hyphomicrobium denitrificans (96.3%) and Hyphomicrobium methyllovorum (96.0%), and as such is distinct enough to define strain CM2¹ as a new species.

Analysis of the 16S rDNA of strain CM4¹ (Fig. 2) showed it to cluster within the Methylobacterium species, as supported by bootstrap values, and is most closely related to Methylobacterium extorquens (98.0% sequence similarity), Methylobacterium rhodesianum (97.6%), Methylobacterium zatmanii (97.8%) and Methylobacterium rhodinum (96.4%). However, strain CM4¹ is distinct from the other Methylobacterium species, being the only Methylobacterium species to grow on chloromethane, and as such is being described as a new species based on DNA–DNA reassociation data (Table 1). None of the eight type strains of Hyphomicrobium species tested [H. facile subsp. facile H-526¹ (DSM 1565), H. facile subsp. ureaphilum CO-582¹ (ATCC 27492), H. facile subsp. tolerans I-551¹ (ATCC 27489), H. vulgare MC-750¹ (ATCC 27500), Hyphomicrobium aestuarii NQ-521¹ (NCIMB 11052), H. denitrificans HA-905, Hyphomicrobium hollandicum KB 677¹ (ATCC 27498), Hyphomicrobium zavarzinii ZV 622¹ (ATCC 27496)] could grow on chloromethane...
as sole carbon and energy source, nor could *M. extorquens*.

### Description of *Hyphomicrobium chloromethanicum* sp. nov.

*Hyphomicrobium chloromethanicum* (chlo.ro.me. tha’ni.cum. N.L. n. *chloromethanicum* chloromethane-utilizing).

Gram-negative, monoprosthecate rods having buds on the prosthecal tips. Cells are 0.5–0.6 µm in diameter and 1.3–1.8 µm long. Cells are motile and non-pigmented. Growth is aerobic. Grows on chloromethane, methanol, methylamine and ethanol as sole carbon and energy sources. C₁-compound assimilation is via the serine pathway, the major quinone is Q₁₀, and the main fatty acid is C₁₈:1ω₃. The optimum temperature for growth is 30°C. The optimum pH for growth is 6.7–7.2. The G+C content of DNA is 64.4 mol%. The GenBank accession number for the 16S rRNA gene sequence of strain CM₄ is AF198624. *M. chloromethanicum* strain CM₄ was isolated from soil at the Nizhnekamsk petrochemical factory, Tatarstan, Russia. The type strain is strain CM₄ and is deposited in the All-Russian Collection of Microorganisms as VKM B-2223 and the National Collections of Industrial, Food and Marine Bacteria as NCIMB 13687.

### Description of *Methylobacterium chloromethanicum* sp. nov.

*Methylobacterium chloromethanicum* (chlo.ro.me. tha’ni.cum. N.L. n. *chloromethanicum* chloromethane-utilizing).

Gram-negative rods 0.8–1.0 µm in diameter and 2.5–3.5 µm long. Cells are motile and pink pigmented.

Growth is strictly aerobic. Grows on chloromethane as sole carbon and energy source. Methanol, methylamine, succinate and fumarate are also growth substrates. Assimilation of C₁ compounds is via the serine pathway, the major quinone is Q₁₀, and the main fatty acid is C₁₈:1ω₃. The optimum temperature for growth is 30°C. The optimum pH for growth is 6.7–7.2. The G+C content of DNA is 64.4 mol%. The GenBank accession number for the 16S rRNA gene sequence of strain CM₄ is AF198624. *M. chloromethanicum* strain CM₄ was isolated from soil at the Nizhnekamsk petrochemical factory, Tatarstan, Russia. The type strain is strain CM₄ and is deposited in the All-Russian Collection of Microorganisms as VKM B-2223 and the National Collections of Industrial, Food and Marine Bacteria as NCIMB 13687.

### DNA–DNA hybridization of *Methylobacterium chloromethanicum* CM₄ with representatives of the genus *Methylobacterium*

DNA–DNA hybridization was carried out according to the method of Denhardt (1966).

<table>
<thead>
<tr>
<th><em>Methylobacterium</em> species</th>
<th>DNA–DNA hybridization (%)</th>
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<tr>
<td><em>M. radiotolerans</em> DSM 1819&lt;sup&gt;T&lt;/sup&gt;</td>
<td>18.2</td>
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<tr>
<td><em>M. organophilum</em> NCIMB 11278&lt;sup&gt;T&lt;/sup&gt;</td>
<td>14.6</td>
</tr>
<tr>
<td><em>M. rhodinum</em> DSM 2163&lt;sup&gt;T&lt;/sup&gt;</td>
<td>19.6</td>
</tr>
<tr>
<td><em>M. extorquens</em> ATCC 43645&lt;sup&gt;T&lt;/sup&gt;</td>
<td>62.8</td>
</tr>
<tr>
<td><em>M. rhodesianum</em> DSM 5587&lt;sup&gt;T&lt;/sup&gt;</td>
<td>21.8</td>
</tr>
<tr>
<td><em>M. zatmanii</em> DSM 5588&lt;sup&gt;T&lt;/sup&gt;</td>
<td>22.6</td>
</tr>
<tr>
<td><em>M. aminovorans</em> ATCC 51358&lt;sup&gt;T&lt;/sup&gt;</td>
<td>47.3</td>
</tr>
<tr>
<td><em>M. mesophilicum</em> ATCC 29983&lt;sup&gt;T&lt;/sup&gt;</td>
<td>14.2</td>
</tr>
<tr>
<td><em>M. fujisawaense</em> NCIMB 12417&lt;sup&gt;T&lt;/sup&gt;</td>
<td>17.4</td>
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**References**

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