NOTE

***Hyphomicrobium chloromethanicum* sp. nov. and *Methylobacterium chloromethanicum* sp. nov., chloromethane-utilizing bacteria isolated from a polluted environment**Ian R. McDonald,¹ Nina V. Doronina,² Yuri A. Trotsenko,²
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e-mail: ip@dna.bio.warwick.ac.uk¹ Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK² Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, 142292 Puschino, Moscow region, Russia**Two chloromethane-utilizing facultatively methylotrophic bacteria, strains CM2^T and CM4^T, were isolated from soil at a petrochemical factory. On the basis of their morphological, physiological and genotypical properties, strain CM2^T (= VKM B-2176^T = NCIMB 13687^T) is proposed as a new species of the genus *Hyphomicrobium*, *Hyphomicrobium chloromethanicum*, and strain CM4^T (= VKM B-2223^T = NCIMB 13688^T) 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 3.5–5 × 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, CM35) and *Methylobacterium* (CM4, CM30, CM34) (Doronina *et al.*, 1996). The *Hyphomicrobium* strains had characteristics typical of the *Hyphomicrobium* type species *Hyphomicrobium vulgare* except for their growth on chloromethane. The cells were rod-shaped, oval or bean-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 CM4^T 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, CM2^T and CM4^T 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 CM2^T and CM4^T.

The DNA of strains CM2^T, CM4^T, 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 CM2^T and CM4^T, 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 CM2^T and CM4^T are AF198623 and AF198624, respectively.

Fig. 1. Phylogenetic analysis of the 16S rRNA sequences of *Hyphomicrobium* sp. strain CM2^T 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.

Computer Group Sequence analysis software package version 8.0 (Genetics Computer Group, Madison, Wisconsin). *Hyphomicrobium* strains CM2^T and CM29 were found to be identical, and *Methylobacterium* strains CM4^T and CM30 were also identical to each other. The complete 16S rRNA gene sequences from strains CM2^T and CM4^T 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, DNAPARS 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 CM2^T (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 CM2^T has high sequence similarity with *Hyphomicrobium facile* subsp. *facile* H-526^T (97.1%), *Hypho-*

Fig. 2. Phylogenetic analysis of the 16S rRNA sequences of *Methylobacterium* sp. strain CM4^T 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.

microbium denitrificans (96.3%) and *Hyphomicrobium methylovorum* (96.0%), and as such is distinct enough to define strain CM2^T as a new species.

Analysis of the 16S rDNA of strain CM4^T (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 CM4^T 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-526^T (DSM 1565), *H. facile* subsp. *ureaphilum* CO-582^T (ATCC 27492), *H. facile* subsp. *tolerans* I-551^T (ATCC 27489), *H. vulgare* MC-750^T (ATCC 27500), *Hyphomicrobium aestuarii* NQ-521^T (NCIMB 11052), *H. denitrificans* HA-905, *Hyphomicrobium hollandicum* KB 677^T (ATCC 27498), *Hyphomicrobium zavarzinii* ZV 622^T (ATCC 27496)] could grow on chloromethane

Table 1. DNA–DNA hybridization of *Methylobacterium chloromethanicum* CM4^T with representatives of the genus *Methylobacterium*

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

<i>Methylobacterium</i> species	DNA–DNA hybridization (%)
<i>M. radiotolerans</i> DSM 1819 ^T	18.2
<i>M. organophilum</i> NCIMB 11278 ^T	14.6
<i>M. rhodinum</i> DSM 2163 ^T	19.6
<i>M. extorquens</i> ATCC 43645 ^T	62.8
<i>M. rhodesianum</i> DSM 5687 ^T	21.8
<i>M. zatmanii</i> DSM 5688 ^T	22.6
<i>M. aminovorans</i> ATCC 51358 ^T	47.3
<i>M. mesophilicum</i> ATCC 29983 ^T	14.2
<i>M. fujisawaense</i> NCIMB 12417 ^T	17.4

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 Q₉ and the main fatty acid is C_{18:1}. The optimum temperature for growth is 28–30 °C. The optimum pH for growth is 6.5–7.5. The G + C content of DNA is 60.0 mol%. *H. chloromethanicum* has 29.4% DNA–DNA hybridization to *H. zavarzinii* ZV-622^T (ATCC 27496). The GenBank accession number for the 16S rRNA gene sequence of strain CM2^T is AF198623. *H. chloromethanicum* strain CM2^T was isolated from soil at the Nizhekamsk petrochemical factory, Tatarstan, Russia. The type strain is strain CM2^T and is deposited in the All-Russian Collection of Microorganisms as VKM B-2176^T, and the National Collections of Industrial, Food and Marine Bacteria as NCIMB 13687^T.

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 C_{18:1}. 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^T is AF198624. *M. chloromethanicum* strain CM4^T was isolated from soil at the Nizhekamsk petrochemical factory, Tatarstan, Russia. The type strain is strain CM4^T and is deposited in the All-Russian Collection of Microorganisms as VKM B-2223^T and the National Collections of Industrial, Food and Marine Bacteria as NCIMB 13688^T.

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