Revised Taxonomy of the Methanotrophs: Description of *Methylobacter* gen. nov., Emendation of *Methylococcus*, Validation of *Methylosinus* and *Methylocystis* Species, and a Proposal that the Family *Methylococcaceae* Includes Only the Group I Methanotrophs

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Numerical taxonomic, DNA-DNA hybridization, and phospholipid fatty acid composition analyses were performed on an extensive range of methanotrophic strains, including reference strains and environmental isolates obtained from sites throughout eastern Australia. When the results of these studies were related to the results of a study based on genomic physicochemical properties, they clarified group I and II methanotroph genus and species interrelationships. The group I methanotrophs were found to be made up of three broadly phenotypically and genotypically homologous clusters of species. The first group I methanotroph cluster included the carotenoid-containing species *Methylomonas methanica*, *Methylomonas fodinarum*, and *Methylomonas aurantiaca*. These species represent the true members of the genus *Methylomonas*. The second group I methanotroph cluster was made up of two subclusters of strains. One subcluster included species not capable of producing resting cells and consisted of the species “*Methylomonas agile*,” “*Methylomonas alba*,” and *Methylomonas pelagica*. The other subcluster included species capable of forming desiccation-resistant cysts and included *Methylococcus luteus*, marine *Methylomonas*-like strains, and *Methylococcus whittenburyi*. Strains designated “*Methylococcus ucrainicus*” and *Methylococcus vinelandii* were found to be synonyms of *Methylococcus whittenburyi*, while *Methylococcus bovis* was a synonym of *Methylococcus luteus*. It is proposed that these subclusters represent a new genus, *Methylobacter* gen. nov. The species in the new genus are type species *Methylobacter luteus* comb. nov., *Methylobacter agilis* sp. nov., *Methylobacter albus* sp. nov., nom. rev., *Methylobacter marinus* sp. nov., *Methylobacter pelagicus* comb. nov., and *Methylobacter whittenburyi* comb. nov. The remaining group I methanotrophs included the moderately thermophilic species *Methylococcus capsulatus* and *Methylococcus thermophilus* and a group of unnamed strains closely related to *Methylococcus capsulatus*. It is proposed that these species represent the true members of the genus *Methylococcus*. The group II methanotrophs consisted of two closely related groups. The first group included budding, exospore-producing strains, while the second group included nonmotile, cyst-forming strains. These groups represent the genera *Methylosinus* and *Methylocystis*, which are revived here. The genus *Methylosinus* gen. nov., nom. rev. includes the species *Methylosinus trichosporium* sp. nov., nom. rev. and *Methylosinus sporium* sp. nov., nom. rev., while the genus *Methylocystis* gen. nov., nom. rev. includes the species *Methylocystis parvus* sp. nov., nom. rev. and *Methylocystis echinoides* sp. nov., nom. rev.

The methanotrophs represent a subset of gram-negative, obligately methylotrophic bacteria that utilize methane as their primary source of carbon and energy. The difficulty in obtaining pure cultures, the lack of discriminating phenotypic characteristics, and transgressions against the *International Code of Nomenclature of Bacteria* (30, 56) have resulted in classification problems in methanotroph taxonomy.

The methanotrophs can be separated into two assemblages, which are usually referred to as types or groups I and II (56). To avoid confusion with such concepts as type strain, type species, etc., in this study we used the terms group I and group II. The group I and II methanotrophs differ in their intracytoplasmic membrane ultrastructures, carbon assimilation

pathways, fatty acid carbon chain lengths (56), and phylogeny (52). In various studies an additional group of methanotrophs, referred to as group X, has also been described (23, 26). Originally, the group X methanotrophs were defined on the basis of G+C values and intracytoplasmic membrane contents (36) and only included some members of the group I methanotroph genus *Methylococcus*. Subsequently, other characteristics have been used to further define this group (26). In this study the term group X was not used to avoid confusion.

Currently, the methanotrophs with valid taxonomic status include only the group I methanotrophs (37) assigned to the genera *Methylococcus* (20) and *Methylomonas* (56). The group II methanotrophs, which include the genera “*Methylocystis*” and “*Methylosinus*” (43, 57), have no standing in bacterial nomenclature (37). Even among the officially recognized genera considerable heterogeneity exists. Genomic characteristics, including G+C values, genome molecular weights, and DNA melting transition widths, separate the

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genus *Methylococcus* into two distinct clusters of species (7). These clusters also contain a number of *Methylomonas* species. One cluster of *Methylococcus* species includes the moderately thermophilic species *Methylococcus capsulatus* (20), *Methylococcus thermophilus*, and "*Methylomonas gracilis*" (35, 43). The G+C values for these species vary from 59 to 66 mol%. The members of the second *Methylococcus* species cluster are mesophilic and have lower G+C contents (range, 48 to 55 mol%) (7, 34–36), and this cluster includes *Methylococcus bovis*, *Methylococcus luteus*, *Methylococcus vinelandii*, and *Methylococcus whittenburyi* (43). Romanovskaya (42) attempted to resolve this heterogeneity by placing the mesophilic low-G+C-content *Methylococcus* species in a different genus, the genus "*Methylovarius*." This genus name is currently invalid (37). The results of DNA-DNA hybridization studies performed by Lysenko et al. (34) suggested that all of these species except *Methylococcus luteus* are closely related. This phenotypic and genotypic ambiguity still remains to be resolved for the species of this group. The species "*Methylomonas alba*" (43) and *Methylomonas pelagica* (45) were also found to be similar to the mesophilic low-G+C-content *Methylococcus* species on the basis of genomic characteristics (7). The remaining *Methylomonas* species, *Methylomonas methanica* (56), *Methylomonas aurantiaca*, and *Methylomonas fodinarum* (6), are distinct in terms of their genomic characteristics (7) and characteristically produce carotenoid pigments. The species "*Methylomonas agile*" (57) appeared to have genomic characteristics similar to those of the carotenoid-containing *Methylomonas* species. However, phenotypically, "*Methylomonas agile*" appears to be more closely related to "*Methylomonas alba*" and does not produce carotenoid pigments.

The results of comparisons of the 5S rRNA sequences of *Methylomonas* and *Methylococcus* species (10, 11) also indicated that the taxonomy of these genera and species requires clarification and revision. For instance, genomic characteristics (7) and the results of 5S rRNA (10) and 16S rRNA sequence analyses (9) suggest that the group II methanotroph genera "*Methylosinus*" and "*Methylocystis*" are closely related but distinctly different groups. However, the phenotypic and genotypic homogeneity among the group II methanotrophs is still to be defined. We found that on the basis of the results of a comprehensive phenotypic survey of several reference methanotroph strains and environmental isolates, accompanied by the results of DNA-DNA hybridization and fatty acid analyses, it was possible to elucidate methanotroph genus and species interrelationships. In this study we established a foundation for an effective systematics for the methanotrophs.

MATERIALS AND METHODS

Bacterial strains and cultivation. The strains investigated in this study are listed in Table 1. These strains were grown under a methane-air-CO₂ (5:4:1) atmosphere. All of the strains except *Methylomonas* sp. strains A1, A4, and C2 and *Methylomonas pelagica* ACM 3505^T (T = type strain) were cultivated on nitrate-mineral salts (NMS) agar (6). *Methylomonas* sp. strains A1, A4, and C1 (33) were grown at 37°C on NMS medium prepared with tap water and supplemented with 0.5% (wt/vol) NaCl and 5 ml of a vitamin solution (50) per liter. *Methylomonas pelagica* ACM 3505^T was cultivated at 25°C on NMS medium prepared with artificial seawater (from Marine agar 2216 [catalog no. 0979; Difco] and solidified with 1.0% (wt/vol) agarose (type V; Sigma Chemical Co., St. Louis, Mo.) (45). The *Methylococcus* strains in

phenon 10 through 12 (Table 1) were cultivated at 45°C, and the other strains in Table 1 were grown at 28°C. The methods used for isolation and purification of methanotrophs have been described previously by Bowman et al. (6). The guidelines described by Lidstrom (33) for determining the purity of methanotroph cultures were followed.

Morphological observations. The Gram stain reaction and the results of the correlative tests, which included aminopeptidase production, lysis in 10% (wt/vol) sodium dodecyl sulfate (SDS), and lysis in 3% (wt/vol) KOH (47), were determined by using 7-day-old cultures. Microcolonies on NMS medium containing Noble agar (catalog no. 0142; Difco) were observed after 24 h by using oil immersion phase-contrast microscopy. The presence of rosettes and exospore formation were verified by this procedure. Capsules were stained with 2.5% (wt/vol) nigrosine (15) and observed by using oil immersion phase-contrast microscopy. Growth from 14-day-old NMS medium cultures was stained by the procedure of Vela and Wyss (53) and with the Ziehl-Neelsen stain (15) and was observed microscopically for the presence of *Azotobacter*-like cysts. The presence of exospores was ascertained by determining the viability of strains after suspensions of 7-day-old NMS medium cultures were heated at 80°C for 20 min by using a heating block (model DB3 Dri-block, Tecam). The presence of exospores was then confirmed by observing microcolonies of the viable and thus heat-resistant strains. Desiccation resistance was assessed by the method described by Whittenbury et al. (55). The presence of poly-β-hydroxybutyrate inclusions was determined chemically (25), and polyphosphate (volutin) was detected by a modification of the method of Neisser (16). Colony morphology and pigmentation were determined by using 7-day-old NMS agar plate cultures.

Electron microscopy. Flagellum insertion was determined by examining negatively stained or metal-shadowed cells with an Hitachi model H-800 transmission electron microscope. The negative stain utilized was phosphotungstate (1%, wt/vol; pH 6.5). The cells were shadowed with 1.7 nm of platinum-carbon from an electron gun at an angle of 30° in a vacuum of approximately 10⁻⁵ torr (133 × 10⁻⁵ Pa), using a high-vacuum coating unit (Balzers Aktiengesellschaft, Fürstentum, Liechtenstein). Thin sections of cells taken from 3-day-old liquid or plate cultures were used to determine intracytoplasmic membrane types. The ruthenium red staining procedures of Fassel et al. (19) and Pierson et al. (40) were used to process the samples.

Physiological tests. The following tests were performed by the rapid titer tray method of Cox and MacRae (13): growth at pHs ranging from 4.0 to 10.0; growth at various temperatures ranging from 4 to 60°C; tolerance to NaCl at concentrations ranging from 0.5 to 5.0% (wt/vol); and tolerance to 0.0075% (wt/vol) KCN, 0.01% (wt/vol) NaN₃, and 0.01% (wt/vol) SDS. The titer trays were incubated under a methane-air (1:1) atmosphere in desiccators. Tolerance to dyes was tested by incorporating dyes into NMS agar plates; the dyes tested included 0.001% crystal violet, 0.001% malachite green, and 0.01% methylene blue.

Carbon and nitrogen source utilization. NMS medium was used as the basal medium to determine utilization of the following carbon sources at a concentration of 0.1%: ethanol, formate, formamide, urea, methanol, methylamine, dimethylamine, trimethylamine, tetramethylammonium chloride, trimethylamine N-oxide, trimethyl N-sulfonium iodide, and dimethylcarbonate. Volatile liquid carbon sources and C₁ compounds were filter sterilized and added after autoclaving to the basal medium, which was then

TABLE 1. Methanotrophic strains investigated in this study

Phenon ^a	Taxon	Strain(s) ^b	Source or isolation site ^{b,c}
1	<i>Methylomonas methanica</i>	ACM 3307 ^T (= S1 ^T = NCIMB 11130 ^T = ATCC 35067 ^T = IMET 10543 ^T) JB5, JB9, JB17, JB227 JB40 JB182, JB193, JB194, JB195 JB228	NCIMB Coal mine drainage water Soil Creek mud River mud NCIMB
2	" <i>Methylomonas rubra</i> "	ACM 3303 (= NCIMB 11913 = VKM-15m)	NCIMB
	<i>Methylomonas fodinarum</i>	JB2, JB3, JB4, JB6, JB7, JB10, ACM 3268 ^T (= JB13 ^T), JB15 JB34, JB37, JB70	Coal mine drainage water Lake sediment
3	<i>Methylomonas aurantiaca</i>	JB25, JB27 JB96, JB97, JB98, JB99, JB100, JB101, JB102, ACM 3406 ^T (= JB103 ^T), JB104, JB105, JB106, JB114, JB121, JB122, JB126 JB129, JB134, JB135, JB142 JB176, JB177a, JB177b, JB178a, JB178b, JB179a, JB179b	Waterlogged soil Sewage sludge and wastewater River mud Lake sediment
4	<i>Methylococcus bovis</i>	IMET 10593	IMET
	<i>Methylococcus</i> sp.	JB50 JB200	Lake sediment Coal mine drainage water
	" <i>Methylococcus ucrainicus</i> "	ACM 3305 (= NCIMB 11915 = VKM-160)	NCIMB
	<i>Methylococcus vinelandii</i>	ACM 3586 ^T (= IMV-B 3030 ^T = VKM-248 ^T)	IMV
	<i>Methylococcus whittenburyi</i>	ACM 3310 ^T (= NCIMB 11128 ^T)	NCIMB
5	<i>Methylomonas</i> sp. (marine)	A1, A45, C1	Lidstrom
6	" <i>Methylomonas agile</i> "	ACM 3308 ^T (= NCIMB 11124 ^T = ATCC 35068 ^T) JB109	NCIMB Sewage
7	" <i>Methylomonas alba</i> "	ACM 3314 ^T (= NCIMB 11123 ^T = IMET 10526 ^T = VKM-BG8 ^T) JB160, JB161 JB162, JB163, JB164, JB166, JB168	NCIMB Soil Creek mud
8	<i>Methylococcus luteus</i>	ACM 3304 ^T (= NCIMB 11914 ^T = VKM-53B ^T) JB107, JB108	NCIMB Sewage
	<i>Methylococcus bovis</i>	IMV-B-3098 ^T (= VKM-6 ^T)	IMV
9	<i>Methylomonas pelagica</i>	NCIMB 2265 ^T	NCIMB
10	<i>Methylococcus capsulatus</i>	ACM 1292 ^T (= ATCC 19069 ^T = NCIMB 11853 ^T) ACM 3302 (= NCIMB 11132 = ATCC 33009 = IMET 10544 = VKM-TRMC) JB115, JB116 JB120, JB137 JB146 JB175	ATCC NCIMB Sewage River mud Marsh mud Lake sediment
11	<i>Methylococcus</i> sp.	JB41, JB43 JB56, JB57 JB83, JB84, JB86, JB87 JB131, JB138, JB139, JB140, JB141, JB143, JB144, JB145, JB173	Creek mud Soil Marsh mud River mud
12	<i>Methylococcus thermophilus</i>	ACM 3585 ^T (= IMV-B 3037 ^T = VKM-2Yu ^T) JB174 JB180, JB187 JB181, JB185	IMV Lake sediment Soil Creek mud
13	" <i>Methylosinus trichosporium</i> "	ACM 3311 ^T (= NCIMB 11131 ^T = ATCC 35070 ^T = IMET 10541 ^T)	NCIMB
14	" <i>Methylosinus sporium</i> "	ACM 3306 ^T (= NCIMB 11126 ^T = ATCC 35069 ^T = IMET 10545 ^T) JB153, JB154, JB159, JB203, JB205, JB206, JB207	NCIMB Rice soil
15	" <i>Methylocystis parvus</i>	ACM 3309 ^T (= NCIMB 11129 ^T = ATCC 35066 ^T = IMET 10483 ^T) JB21 JB22, JB47, JB48, JB51, JB52 JB29, JB30 JB33 JB190 JB191, JB192, JB196, JB198	NCIMB Coal mine drainage water Soil Raw water Lake sediment Marsh mud Creek mud
16	" <i>Methylocystis echinoides</i> "	IMET 10491 ^T	IMET

^a See Fig. 1 for location on dendrogram.^b Abbreviations: ACM, Australian Collection of Microorganisms, The University of Queensland, Brisbane, Australia; ATCC, American Type Culture Collection, Rockville, Md.; IMET, Institute of Medical and Experimental Therapy, Jena, Germany; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, United Kingdom. VKM, All-Union Culture Collection of Microorganisms, Academy of Sciences, Moscow, Russia; IMV, V. A. Romanovskaya, Institute of Microbiology and Virology, Academy of Sciences, Kiev, Ukraine; Lidstrom, M. E. Lidstrom, California Institute of Technology, Pasadena, Calif.^c The methods used for enrichment and isolation of environmental methanotrophic isolates are described in reference 6.

dispensed in 10-ml volumes into screw-cap test tubes (Wheaton, Inc., Millville, N.J.). The following carbon sources were tested by the rapid titer tray method (13): D-arabinose, D-xylose, D-fructose, D-glucose, acetate, DL-

lactate, sebacate, DL-tartrate, citrate, L-aspartate, L-glutamate, and betaine. Controls lacking a carbon substrate and nutrient broth controls were included to guard against the possibility that mixed methanotroph cultures might cause

false-positive results. Nitrogen sources were tested similarly by using NMS medium in which the sodium nitrate was replaced with one of the following compounds at a concentration of 0.1%: sodium nitrite, ammonium sulfate, hydroxylamine hydrochloride, glycine, DL-alanine, L-serine, L-threonine, L-valine, L-leucine, L-isoleucine, L-proline, L-cysteine, L-methionine, L-glutamate, L-aspartate, L-glutamine, L-asparagine, L-histidine, L-phenylalanine, L-tryptophan, L-arginine, L-lysine, L-ornithine, L-citrulline, 2-aminobutyrate, 4-aminobutyrate, 2-aminovalerate, 5-aminovalerate, putrescine, spermine, histamine, ethanolamine, α -amylamine, betaine, creatine, hippurate, yeast extract, and Casamino Acids. Utilization of methylamine, dimethylamine, trimethylamine, trimethylamine N-oxide, tetramethylammonium chloride, trimethylsulfonium iodide, and formamide as nitrogen sources was tested in test tubes instead of titer trays. All test preparations were incubated for 30 days in duplicate. Growth was confirmed by comparison with negative controls and by spectrophotometry at A_{600} . The titer trays were examined by using a Titertek titer tray reader at 600 nm.

Biochemical tests. Catalase and oxidase activities were determined as described by Smibert and Krieg (47). Nitrate reduction and denitrification activity were tested in semisolid NMS medium containing 0.2% (wt/vol) KNO_3 . The semisolid medium was dispensed into test tubes (10 ml per tube); each tube also contained an inverted Durham tube. Nitrite was detected by diazotization (25). Nitrogen fixation was assayed by using the modified acetylene reduction procedure of Takeda (51). Hydrogen autotrophy was determined by the procedure of Sly (46), using liquid and semisolid NMS media as the growth media. Urease production was determined by observing alkalization of NMS medium supplemented with 0.2% (wt/vol) urea and 0.005% (wt/vol) phenol red. Thiosulfate:cyanide sulfurtransferase activity (rhodanese test) was assayed by the procedure described by Lanyi (29). The presence of phosphatase and the presence of arylsulfatase were determined by the method of Jenkins and Jones (28), except that the basal medium used was NMS medium. Acid production from D-glucose was tested by supplementing NMS medium with 1.0% (wt/vol) glucose and 0.005% (wt/vol) bromothymol blue. Indole production from tryptophan was tested by supplementing NMS medium with 0.1% (wt/vol) L-tryptophan and using Ehrlich's reagent (4-dimethylaminobenzaldehyde in 70% [vol/vol] ethanol, 1.5 M HCl) (29). Esculin hydrolysis was determined by supplementing liquid NMS medium with 0.1% (wt/vol) esculin and 0.005% (wt/vol) ferric citrate (47). Reduction of 2,3,5-triphenyl-2H-tetrazolium chloride (0.01%, wt/vol), reduction of potassium tellurite (0.1%, wt/vol), and reduction of sodium selenite (0.1%, wt/vol) were determined in NMS agar.

Enzyme assays. Selected methanotroph isolates were grown on NMS medium for 3 to 4 days, harvested into 10 mM Tris (pH 7.0) buffer, and then washed twice by centrifugation at $10,000 \times g$ for 10 min at 4°C . The cells, chilled in ice, were disrupted by three to five 20-s pulses of ultrasonication with a model 250 Sonifier (Branson, Danbury, Conn.) at the maximum power setting (250 kHz). The cell debris was removed by centrifugation at $20,000 \times g$ for 30 min at 4°C . The activities of the following enzymes were assayed by using the resulting cell extract: hydroxypyruvate reductase (EC 1.1.1.29) (31), hexulose phosphate synthase (14), hydroxylamine cytochrome c oxidoreductase (EC 1.7.3.4) (49), and ribulose-1,5-diphosphate carboxylase (EC 4.1.1.39) (1). The assays were performed with a model DU-8 spectrophotometer (Beckman Instruments, Fullerton, Calif.) at 25°C .

Numerical taxonomy. Results were encoded in a binomial format (41). Data were analyzed by using the Taxan program (version 3.0) (Information Resources Group, Maryland Institute of Biotechnology, University of Maryland, College Park) and an IBM-compatible personal computer. Jaccard coefficients (S_j) were used, and a similarity matrix was prepared by the unweighted pair group average procedure (48).

DNA-DNA hybridization analysis. DNAs were extracted from methanotrophs by procedures described by Bowman et al. (7). The spectrophotometric thermal renaturation procedure of Huss et al. (27) was used to determine levels of DNA homology between selected strains. G+C values determined from the optimum renaturation temperatures were calculated as described by Bowman et al. (7).

PLFA profiles. To determine phospholipid fatty acid (PLFA) profiles, the total lipids of lyophilized cell material were extracted and fractionated with activated silicic acid (24, 54). The phospholipid fraction was transesterified to the corresponding methyl esters by using acid methanolysis and was analyzed by high-resolution gas chromatography (39). Fatty acid isomers and double-bond positions were determined by dimethyldisulfide derivitization (17, 38) and analysis by gas chromatography-mass spectrometry.

RESULTS

Numerical analysis. A total of 124 characters for 136 methanotrophic strains were included in the numerical analysis. When a cutoff similarity value (S_j) of $\geq 72\%$ was used to group the strains (Fig. 1), a total of 16 phena were formed. The assignments of individual strains to phena are shown in Table 1. The phenotypic results obtained for each of the phena are shown in Table 2. Tentative identification of many environmental isolates could be made as these isolates were found to be very similar to reference strains. The group I methanotrophs were divided into phena 1 to 12, while the group II methanotrophs were divided into phena 13 to 16. Broader groups of phena were also discernable at a similarity value of 50% (Fig. 1).

The group I methanotrophs formed three such groups. The first of these included phena 1 to 3 and contained the pink and orange carotenoid-containing species *Methylomonas methanica*, *Methylomonas fodinarum*, and *Methylomonas aurantiaca*, respectively. These species were closely allied phenotypically, clustering together at an S_j value of $\geq 69\%$ (Fig. 1), and had the following general properties: coccobacillary or rod-shaped cells which were encapsulated, contained poly- β -hydroxybutyrate inclusions, non-desiccation-resistant cysts, and carotenoid pigments, and were motile by means of a single polar flagellum. More detailed phenotypic traits that distinguish these species are shown in Table 2.

A greater degree of phenotypic diversity was found in the second large group, which encompassed phena 4 to 9 (which clustered together at a similarity level of $\geq 55\%$). A number of desiccation-resistant, cyst-producing (55) *Methylococcus* species were clustered in this group, including "*Methylococcus ucrainicus*" (phenon 4), *Methylococcus vinelandii* (phenon 4), *Methylococcus whittenburyi* (phenon 4), *Methylococcus bovis* (phenon 8), and *Methylococcus luteus* (phenon 8). Closely clustering with phenon 4 at an S_j value of $\geq 64\%$, phenon 5 contained three *Methylomonas*-like strains (strains A1, A4, and C1) which were isolated from seawater by Lidstrom (33) and were also capable of forming desiccation-resistant cysts. These strains differed from phenon 4 strains in that they required NaCl for growth and also required an

FIG. 1. Simplified dendrogram based on the results of a numerical analysis of 124 phenotypic characteristics of 136 methanotrophic bacteria. S_J values were generated and clustered by using the unweighted pair group average procedure and the Taxan computer program. n is the number of strains in a cluster.

unidentified component present in tap water (33); otherwise, phenon 5 strains were morphologically and biochemically similar to phenon 4 strains (Table 2). In addition to the desiccation-resistant cyst-forming *Methylococcus* species, a number of *Methylomonas* species were also present in the

second broad group (phena 6, 7, and 9). These strains differed from the *Methylococcus* species in phena 4, 5, and 8, having a more rodlike shape, and completely lacked a differentiated encystment stage. Phena 6, 7, and 9 included the species "*Methylomonas agile*," "*Methylomonas alba*,"

TABLE 2. Phenotypic characteristics of methanotrophic phena defined by numerical analysis

Characteristic	Phenon 1: <i>Methy- lomonas methanica</i> (n = 13) ^a	Phenon 2: <i>Methy- lomonas fodi- narum</i> (n = 11)	Phenon 3: <i>Methy- lomonas auran- tiaca</i> (n = 28)	Phenon 4: <i>Methy- lomonas spp.</i> (n = 6)	Phenon 5: <i>Methy- lomonas spp.</i> (n = 3)	Phenon 6: "Me- thy- lomonas agie" (n = 2)	Phenon 7: "Me- thy- lomonas alba" (n = 8)	Phenon 8: <i>Methy- lomonas luteus</i> (n = 4)	Phenon 9: <i>Methy- lomonas pelagica</i> (n = 1)	Phenon 10: <i>Methy- lomonas capsu- latus</i> (n = 8)	Phenon 11: <i>Methy- lomonas sp.</i> (n = 7)	Phenon 12: <i>Methy- lomonas thermo- philus</i> (n = 7)	Phenon 13: "Me- thy- lomonas trichos- porium" (n = 1)	Phenon 14: "Me- thy- lomonas sporium" (n = 7)	Phenon 15: "Me- thy- lomonas cystis parvus", echinoides" (n = 16)	Phenon 16: "Me- thy- lomonas cystis parvus", echinoides" (n = 1)
Cell morphology																
Cocci	- ^b	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
Ellipsoidal	+	+	+	+	+	50	25	50	-	-	12	43	-	-	-	-
Rods	+	+	+	33	+	+	+	-	+	50	+	+	-	+	+	+
Coccibacilli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reniform or vibrioid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pyriform	77	+	+	-	67	50	37	-	+	50	-	-	-	-	-	-
Length 0.5-1.0 µm	92	+	+	+	+	+	+	+	+	75	+	+	+	62	+	+
Length 2.1-3.0 µm	-	-	-	33	-	-	-	-	-	-	-	-	-	75	-	-
Length 2.1-3.0 µm	8	-	-	-	-	50	-	-	-	-	-	-	-	-	-	-
Width <0.5 µm	92	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Width 0.6-1.0 µm	+	+	+	+	+	50	+	+	+	+	+	+	+	+	+	+
Poly-β-hydroxybutyrate	+	+	+	+	+	+	87	+	+	50	+	43	-	-	-	-
Polyphosphate	+	+	+	17	67	-	-	+	-	+	+	+	-	-	-	-
Cyst formation	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+
Exospore formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Type II intracytoplasmic membrane	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Type II intracytoplasmic membrane	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
Capsule	69	+	+	+	+	+	50	25	-	+	-	+	+	+	+	+
Motility (1 polar flagellum)	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-
Motility (>1 polar flagella)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spinae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chain formation	23	+	+	-	-	-	-	25	-	-	-	-	-	-	-	-
Colony morphology																
Translucent	-	-	-	-	-	+	13	-	-	-	-	-	-	-	-	-
Opaque	+	+	+	67	+	-	87	+	+	+	+	+	+	+	+	+
Low convex	38	+	93	-	+	+	+	+	+	+	82	14	+	+	+	+
High convex	+	+	82	-	-	50	-	-	-	-	14	86	+	+	+	+
Entire edges	+	+	+	+	+	50	66	50	+	+	-	-	+	+	+	+
Lobate or irregular edges	-	-	-	-	-	50	+	+	-	-	88	14	+	+	+	+
Butyrous consistency	62	-	-	17	+	+	+	25	-	-	12	-	+	+	+	+
Viscid or mucoid consistency	62	+	79	+	-	-	-	-	-	-	-	-	-	-	-	-
Cartilaginous consistency	38	-	61	-	-	-	-	-	38	-	61	-	-	-	-	-
Pigmentation																
White or buff	-	-	-	+	67	+	+	-	+	+	+	+	+	+	26	+
Brown	-	-	-	-	33	-	-	-	-	-	-	-	-	-	62	-
Pink or red	+	+	+	-	-	-	-	-	-	-	-	-	-	12	12	-
Orange	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yellow	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Brown (diffusible)	-	-	-	+	+	-	-	-	-	-	-	+	-	37	62	-
Yellow (diffusible)	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Carotenoids	+	+	+	-	-	-	-	25	-	-	-	-	-	-	-	-

TABLE 2—Continued

Characteristic	Phenon 1: <i>Methy- lomonas methanica</i> (n = 13) ^a	Phenon 2: <i>Methy- lomonas fodi- narum</i> (n = 11)	Phenon 3: <i>Methy- lomonas auran- tiaca</i> (n = 28)	Phenon 4: <i>Methy- lococcus spp.</i> (n = 6)	Phenon 5: <i>Methy- lomonas spp.</i> (n = 3)	Phenon 6: "Me- thy- lomonas agile" (n = 2)	Phenon 7: "Me- thy- lomonas alba" (n = 8)	Phenon 8: <i>Methy- lococcus luteus</i> (n = 4)	Phenon 9: <i>Methy- lomonas pelagica</i> (n = 1)	Phenon 10: <i>Methy- lococcus capsu- latus</i> (n = 8)	Phenon 11: <i>Methy- lococcus spp.</i> (n = 7)	Phenon 12: <i>Methy- lococcus thermo- philus</i> (n = 7)	Phenon 13: "Me- thy- lomonas trichos- porum" (n = 1)	Phenon 14: "Me- thy- lomonas sportum" (n = 7)	Phenon 15: "Me- thylo- cystis parvus" (n = 16)	Phenon 16: "Me- thyl- cystis echinoides" (n = 1)
Trimethylamine-N-oxide	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nitrogen source utilization	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nitrite	8	—	93	67	—	50	75	—	—	—	—	71	—	—	—	—
Hydroxylamine	—	+	97	67	—	50	37	—	—	+	—	86	+	—	6	—
L-Alanine	—	—	—	—	—	—	87	+	—	+	—	—	+	+	+	—
DL-2-Aminobutyrate	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DL-4-Aminobutyrate	—	+	+	—	—	—	—	50	—	+	—	—	—	—	19	—
DL-2-Aminovalerate	—	—	46	17	—	—	37	—	—	—	—	—	—	—	—	—
DL-5-Aminovalerate	—	—	—	—	—	—	87	+	—	+	—	—	+	—	—	—
L-Arginine	—	+	+	—	33	—	—	—	—	+	—	—	—	87	+	—
L-Asparagine	—	+	+	+	+	+	+	+	—	+	+	—	—	+	+	+
L-Aspartate	—	+	+	+	+	—	+	+	+	+	+	—	—	+	+	+
L-Citrulline	—	+	39	—	—	—	—	50	—	+	+	—	—	+	75	+
L-Cysteine	—	—	—	—	—	—	62	—	—	—	—	—	—	—	—	—
L-Glutamine	—	+	+	+	+	+	50	+	—	+	+	—	—	+	+	+
Hippurate	—	—	—	67	—	—	+	—	—	75	+	—	—	—	—	—
L-Histidine	—	36	89	—	—	—	—	—	—	50	—	—	—	—	—	—
L-Lysine	—	—	—	—	—	—	—	—	—	—	35	—	+	75	+	+
L-Methionine	31	82	86	—	—	—	—	—	—	—	—	—	—	—	—	—
L-Ornithine	—	82	89	—	—	—	—	—	—	—	—	—	+	87	87	—
L-Proline	—	+	—	+	+	—	+	75	—	+	+	—	+	75	+	+
L-Tryptophan	—	—	—	33	—	—	87	—	—	—	82	—	—	37	+	+
Ethanolamine	77	—	+	—	—	+	—	25	—	—	—	—	—	—	—	—
Histamine	—	36	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Methylamine	+	+	+	—	67	+	87	50	—	75	+	+	—	75	—	—
Dimethylamine	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Putrescine	+	+	61	—	—	—	+	—	—	+	+	—	+	87	75	—
Formamide	—	—	—	—	—	—	+	—	—	—	—	86	—	—	56	—

^a See Table 1 for species and strain compositions of phena.

^b +, 100% of the strains are positive; —, none of the strains positive. The values indicate the percentages of strains that are positive. The following characteristics were positive for all strains: gram-negative staining reaction; solubility in 10% SDS and 3% KOH; aminopeptidase activity; growth between pH 6.0 and 8.5; hydroxylamine cytochrome c oxidoreductase activity; catalase activity; utilization of methane and methanol as sole carbon and energy sources; and utilization of ammonium sulfate, nitrate, yeast extract, Casamino Acids, and L-glutamate as sole nitrogen sources. The following characteristics were negative for all of the strains tested: growth on nutrient agar; hydrogen autotrophy; denitrification; arylsulfatase activity; acid production from D-glucose; indole production from L-tryptophan; rhodanese; reduction of tellurite; utilization of trimethyl N-sulfonium iodide, tetramethylammonium chloride, N,N-dimethylformamide, urea, and compounds with carbon-carbon bonds as sole sources of carbon and energy; and utilization of trimethylamine, trimethylamine N-oxide, trimethyl N-sulfonium iodide, tetramethylammonium chloride, glycine, L-serine, L-threonine, L-valine, L-leucine, L-isoleucine, L-phenylalanine, L-tyrosine, spermine, betaine, creatine, and α-amylamine as nitrogen sources.

and *Methylomonas pelagica*, respectively. The first two of these species were found to be relatively similar phenotypically, clustering together at an S_j value of $\geq 67\%$ and differing primarily in their carbon and nitrogen source utilization spectra (Table 2). On the other hand, *Methylomonas pelagica* (phenon 9) required seawater for growth and was substantially more biochemically and nutritionally inert (Table 2).

The final broad group (phena 10 to 12) making up the group I methanotrophs included spherical and coccibacillary cells which produced desiccation-sensitive cysts. These strains were moderately thermophilic, with optimal growth temperatures ranging from 40 to 55°C. Phenon 10 contained various strains of *Methylococcus capsulatus*, while phenon 11, which clustered with phenon 10 at an S_j value of $\geq 67\%$, contained 17 environmental isolates isolated from creek, river, and marsh mud, as well as from soil (Table 1). Interestingly, phenon 11 appeared to represent a distinct taxon of methanotrophic bacteria allied to but phenotypically distinct from *Methylococcus capsulatus*. This conclusion was supported by the significant phenotypic coherence observed among the strains in phenon 11 (S_j , $\geq 87\%$). Phena 10 and 11 were the most versatile nutritionally of all the methanotrophs studied, and they could be readily distinguished on the basis of the results of biochemical and carbon and nitrogen source tests (Table 2). Clustering with phena 10 and 11 at an S_j value of $\geq 54\%$, phenon 12 included the species *Methylococcus thermophilus* and "*Methylomonas gracilis*." All of the phenon 12 strains, which clustered together at an S_j value of $\geq 80\%$, were brown pigmented with a brown diffusible pigment produced when the organisms were first isolated. This pigment was often lost rapidly during the purification process. Morphologically and biochemically, the phenon 12 strains appeared to be similar to the phenon 10 and 11 strains except that the phenon 12 strains were motile by means of a single polar flagellum and were comparatively nutritionally inert (Table 2).

The group II methanotrophs clustered as one large group at a similarity level (S_j) of $\geq 53\%$. Phenon 13 strain "*Methylosinus trichosporium*" ACM 3311 and phenon 14 strains ("*Methylosinus sporium*" ACM 3306 and related environmental strains) clustered at a similarity value (S_j) of $\geq 65\%$ on the dendrogram (Fig. 1). All "*Methylosinus*" strains budded off exospores (57) and were motile by means of a tuft of flagella inserted at one pole. "*Methylosinus trichosporium*" ACM 3311 could be differentiated from "*Methylosinus sporium*" strains, which grouped together in phenon 12 at an S_j value of $\geq 93\%$, by its pyriform rather than vibrioid morphology, by its lack of a brown diffusible pigment or red pigment, by its lack of urease and alkaline phosphatase activities, and by various differences in nitrogen source usage (Table 2). Phenon 15 included "*Methylocystis parvus*" ACM 3309, *Methylocystis minimus*" ACM 3497, and 14 environmental isolates clustering together at an S_j value of $\geq 76\%$. Phenon 15 was related to "*Methylocystis echinoides*" ACM 3495 (phenon 16) at an S_j value of $\geq 66\%$. The "*Methylocystis*" strains had the following properties: vibrioid or reniform cells that were small (0.5 to 1.0 by 0.5 to 0.7 μm), lacked motility, and formed lipid cysts (55). "*Methylocystis echinoides*" (22) strain ACM 3495 could be distinguished from phenon 13 strains by its production of unusual surface protuberances (or spinae) (18), by its lack of pigmentation, by its inability to grow at 37°C or below pH 6.0, and by its lack of urease or phosphatase production (Table 2).

DNA-DNA hybridization. Levels of DNA homology were determined between representatives of each of the phena identified in the numerical analysis (Table 3). Levels of genetic relatedness between the species in phena 1 through 3 have been reported previously (6). "*Methylomonas rubra*" ACM 3303 was 100% related to *Methylomonas methanica*. *Methylomonas fodinarum* and *Methylomonas aurantiaca* were also closely related, exhibiting DNA homology levels of $45\% \pm 15\%$; however, these organisms exhibited no significant level of homology with any of the *Methylomonas methanica* strains tested. High levels of DNA homology were detected between strains clustered in phenon 4 and between strains clustered in phenon 8. The phenon 4 species "*Methylococcus ucrainicus*," *Methylococcus vinelandii*, and *Methylococcus whittenburyi* exhibited DNA homology levels of 54 to 93%, while the phenon 8 species *Methylococcus bovis* and *Methylococcus luteus* exhibited a level of homology of 98%. The strains in phena 5 through 7 and 9 were found to be genetically distinct (Table 3). *Methylococcus capsulatus* (phenon 10) exhibited significant levels of DNA relatedness (40 to 43%) to the strains in phenon 11. The phenon 11 strains formed a genetically homologous group, with five test strains exhibiting levels of homology of 63 to 103% with reference culture JB140 (Table 3). *Methylococcus thermophilus* ACM 3585^T exhibited 68% DNA homology with the phenotypically related species "*Methylomonas gracilis*" (both members of phenon 12); however, no significant level of homology was detected between these species and strains in phena 10 and 11. The group II methanotroph phena (phena 13 through 16) were genetically distinct (Table 4).

PLFAs. By using data from this study and other methanotroph PLFA studies (3, 5, 24, 44) it was possible to divide the group I methanotrophs into six groups on the basis of PLFA profiles, while the group II methanotrophs formed a single group (Table 5). The first profile was found in *Methylomonas methanica*, *Methylomonas fodinarum*, and *Methylomonas aurantiaca* (phena 1 through 3); the dominant PLFA components were 14:0 (19 to 25%), 16:1 ω 8c (26 to 41%), and 16:1 ω 5t (8 to 15%). The second profile was found in *Methylococcus* species which produced desiccation-resistant cysts (e.g., phena 4, 5, and 8); these species contained mainly 16:1 ω 7c (57%). The third profile was found in "*Methylomonas agile*" (phenon 6), "*Methylomonas alba*" (phenon 7), and *Methylomonas pelagica* (phenon 9), and the major PLFA components found were 16:1 ω 8c (12 to 19%), 16:1 ω 7c (14 to 20%), 16:1 ω 6c (6 to 14%), 16:1 ω 5t (6 to 28%), and 16:0 (11 to 18%). *Methylococcus capsulatus* strains (phenon 10) contained primarily 16:0 (33 to 56%); significant levels of 16:1 ω 7c (11 to 23%) and 16:1 ω 6c (4 to 12%) were also present. The PLFA profile of phenon 11 strains was found to be quite similar to that of *Methylococcus capsulatus*; however, the levels of 18:1 ω 7c (9 to 15%) and cy19:0 (8 to 16%) were significantly greater (Table 5). The PLFA profiles of *Methylococcus thermophilus* and "*Methylomonas gracilis*" were also somewhat similar to the profiles of the phenon 10 and 11 strains examined; however, the 16:1 isomers were completely dominated by 16:1 ω 7c (17 to 46%), which in most of the strains was present at nearly the same level as 16:0 (40 to 47%). The group II methanotroph PLFA profile was characterized by its high content of the unusual PLFA 18:1 ω 8c (53 to 74%), while 18:1 ω 7c (15 to 38%) was also present at significant levels.

TABLE 3. Levels of DNA hybridization between group I methanotrophs

Strain	Phenon	% DNA homology ^a to:										
		<i>Methy- lomonas methanica</i> ACM 3307 ^T	<i>Methy- lomonas fodinarium</i> ACM 3268 ^T	<i>Methy- lomonas aurantiaca</i> ACM 3406 ^T	<i>Methylo- coccus whitten- buryi</i> ACM 3310 ^T	<i>Methy- lomonas</i> sp. strain A45	<i>"Methylo- monas agile"</i> ACM 3308 ^T	<i>"Methylo- monas alba"</i> ACM 3314 ^T	<i>Methylo- coccus luteus</i> ACM 3304 ^T	<i>Methylo- coccus capsulatus</i> ACM 3302	<i>Methylo- coccus</i> sp. strain JB140	<i>Methylo- coccus ther- mophilus</i> ACM 3585 ^T
<i>Methylomonas methanica</i> ACM 3307 ^T	1	100	25	20	15	12	12	20	11			
<i>Methylomonas methanica</i> JB17	1	68 ^a										
<i>Methylomonas methanica</i> JB193	1	92										
<i>"Methylomonas rubra"</i> ACM 3303	1	100	5									
<i>Methylomonas fodinarum</i> ACM 3268 ^T	2	25	100	47			16	12				
<i>Methylomonas fodinarum</i> JB34	2	6	90	61								
<i>Methylomonas fodinarum</i> JB70	2	10	87	50								
<i>Methylomonas fodinarum</i> JB27	2	2	30	83								
<i>Methylomonas aurantiaca</i> JB103	3	20	47	100		10	9		7			
<i>Methylomonas aurantiaca</i> JB129	3	17	46	98								
<i>Methylomonas aurantiaca</i> JB177a	3		57	95								
<i>Methylococcus</i> sp. strain ACM 3504	4				82				32			
<i>Methylococcus</i> sp. strain JB50	4				93							
<i>Methylococcus</i> sp. strain JB200	4				84							
<i>"Methylococcus ucrainicus"</i> ACM 3305	4	16			54	20	5	17	29			
<i>Methylococcus vinelandii</i> ACM 3586 ^T	4	17			78	27			26			
<i>Methylococcus whittenburyi</i> ACM 3310 ^T	4	15			100	39	16	25	30	10		16
<i>Methylomonas</i> sp. strain A1	5					103						
<i>Methylomonas</i> sp. strain A45	5	12	6		39	100	15	12	20	4		8
<i>Methylomonas</i> sp. strain C1	5				22	81						
<i>"Methylomonas agile"</i> ACM 3308 ^T	6	12	16	10	16	15	100	29	25	14		8
<i>"Methylomonas alba"</i> ACM 3314 ^T	7	20	12	9	25	11	29	100	14			
<i>"Methylomonas alba"</i> JB163	7				11			92	98			
<i>Methylococcus bovis</i> IMV-B-3098 ^T	8	8			21			11	100			
<i>Methylococcus luteus</i> ACM 3304 ^T	8	11			30	20		25	78			
<i>Methylococcus luteus</i> JB107	8											
<i>Methylomonas pelagica</i> ACM 3505 ^T	9	19			7	18		13	9			
<i>Methylococcus capsulatus</i> ACM 1292 ^T	10			7	10	4	14			88	43	20
<i>Methylococcus capsulatus</i> ACM 3302	10								100	40		27
<i>Methylococcus capsulatus</i> JB115	10								72			
<i>Methylococcus capsulatus</i> JB137	10								93			
<i>Methylococcus</i> sp. strain JB41	11											
<i>Methylococcus</i> sp. strain JB57	11											
<i>Methylococcus</i> sp. strain JB87	11									63	70	
<i>Methylococcus</i> sp. strain JB138	11									36	97	
<i>Methylococcus</i> sp. strain JB140	11									23	103	11
<i>Methylococcus</i> sp. strain JB145	11									43	100	
<i>Methylococcus thermophilus</i> ACM 3585 ^T	12									45	85	7
<i>Methylococcus thermophilus</i> JB185	12									20	11	100
<i>Methylomonas gracilis</i> ACM 3315	12		22	17	16	8	8			27	18	68

^a DNA homology value is the average of three determinations. Some of the other data are from reference 6.

TABLE 4. Levels of DNA hybridization between group II methanotrophs

Strain	Phenon	% DNA homology ^a to:			
		" <i>Methylosinus trichosporium</i> " ACM 3311 (phenon 13)	" <i>Methylosinus sporium</i> " ACM 3306 (phenon 14)	" <i>Methylocystis parvus</i> " ACM 3309 (phenon 15)	" <i>Methylocystis echinoides</i> " ACM 3495 (phenon 16)
" <i>Methylosinus trichosporium</i> " ACM 3311 ^T	13	100	21	19	9
" <i>Methylosinus sporium</i> " ACM 3306 ^T	14	21	100	16	21
JB153	14		90		
JB206	14	10	71	12	
" <i>Methylocystis minimus</i> " ACM 3497	15	16		68	
" <i>Methylocystis parvus</i> " ACM 3309 ^T	15	8	7	100	20
JB33	15		3	96	
JB170	15		12	101	
JB190	15			78	
JB196	15		14	82	
" <i>Methylocystis echinoides</i> " ACM 3495 ^T	16	9		20	100

^a DNA homology value is the average of three determinations.

DISCUSSION

On the basis of the results of an extensive phenotypic survey it was possible to elucidate methanotroph species and genus relationships. The results of DNA-DNA hybridization analyses indicate that several species synonyms exist in the current nomenclature. Using numerical analysis, PLFA compositions, and genomic characteristics (7) together, we were able to clarify the species and genera of the group I and II methanotrophs.

Unsuccessful attempts were made to obtain cultures of the taxonomically valid methanotrophs *Methylococcus chroococcus* and *Methylococcus mobilis*. Several other methanotrophs, all invalid and usually based on only limited descriptions from relatively cursory examinations, were also not included in this study. Most of these species have no extant cultures or designated type or reference cultures, while others are simply synonyms. The review of methanotroph systematics by Green (23) covers some of these taxa, including "*Methylococcus minimus*," *Methylococcus mobilis*, "*Methylocystis methanolicus*," "*Methylocystis pyreiformis*," "*Methylomonas flagellata*," "*Methylomonas margaritae*," "*Methylomonas rosaceus*," "*Methylomonas streptobacterium*," "*Methylovibrio soehngenii*," and "*Soehngenella thermomethanica*." Other "obscure" methanotrophs include "*Echinosporeobacterium album*" and "*Polysporobacterium arenarium*" (32).

Family *Methylococcaceae*. On the basis of 16S rRNA phylogeny, the group I methanotroph genera collectively form a relatively distinct branch in the gamma subdivision (8); in addition, the group I methanotrophs share several features which differ from the features of the group II methanotrophs. The current description of the family *Methylococcaceae* includes all of the methanotrophs and the genus *Methylobacterium* (56). It is proposed that the family *Methylococcaceae* should include only the group I methanotrophs, with the genus *Methylococcus* as the type genus. An emended description of the family *Methylococcaceae* is given below.

Emended description of the family *Methylococcaceae* (ex Whittenbury and Krieg 1984). Cell morphology varies from rods to cocci that are 0.5 to 1.5 μ m in diameter and 1.0 to 3.0 μ m long. Cells usually occur singly or in pairs, occasionally forming chains. Cells do not form rosettes. Gram negative. The majority of the species produce a dormant resting stage that appears as a cyst similar to the cysts which occur in members of the genus *Azotobacter*. Cysts may be desiccation resistant or sensitive. If the cells are motile, they

possess a single polar flagellum. Catalase is produced. Strictly aerobic chemoheterotrophs. Obligate utilizers of C₁ compounds (primarily methane and methanol) for carbon and energy. A few species can utilize methylated amines, formate, or formamide. Compounds with carbon-carbon bonds are not utilized. C₁ compounds are incorporated via the ribulose monophosphate pathway. The tricarboxylic acid pathway is usually incomplete, lacking 2-oxoglutarate dehydrogenase. The dissimilatory methane oxidation pathway is associated with type I intracytoplasmic membranes which appear as stacks of vesicular discs. No growth occurs on complex organic media. No growth factors are required. Nitrogen is fixed by an oxygen-sensitive nitrogenase (not present in *Methylobacter* strains). Denitrification does not occur. Ammonia is oxidized to nitrite nonspecifically via methane monooxygenase and hydroxylamine cytochrome c oxidoreductase. The G+C content ranges from 49 to 66 mol% (point of temperature inflection, T_i). The genome molecular weight varies from 1.5 $\times 10^9$ to 3.0 $\times 10^9$. Most fatty acids have a carbon chain length of 16 and are either saturated or monounsaturated; several different 16:1 isomers are present in some species. Ubiquinone 8 or its 18-methylated version (MQ-8) are the predominant lipoquinones present (12). The member genera include *Methylomonas*, *Methylobacter*, and *Methylococcus*, which form a cluster within the γ subdivision of the *Proteobacteria* and are collectively referred to as the group I methanotrophs. The type genus is the genus *Methylococcus*.

Genus *Methylomonas*. The first group I methanotroph species cluster consists of *Methylomonas methanica*, *Methylomonas fodinarum*, and *Methylomonas aurantiaca*. A numerical analysis based both on S_j values (Fig. 1) and simple matching coefficients (6) showed that these species are distinct from other group I methanotrophs. PLFA composition was highly consistent among the species (Table 5) (5). The genomic physicochemical characteristics, including G+C values (51 to 59 mol%), genome molecular weights (1.8 $\times 10^9$ to 2.3 $\times 10^9$), and DNA melting transition widths (10.5 to 12.0 mol% G+C), also indicate that these species are similar (7). The 16S rRNA sequences indicate that *Methylomonas methanica* forms a branch in the γ subdivision of the *Proteobacteria* quite distinct from *Methylococcus capsulatus* (8, 9, 52). It is proposed that the species *Methylomonas methanica*, *Methylomonas aurantiaca*, and *Methylomonas fodinarum* are the true members of the genus *Methylomonas*. These species are differentiated in Table 6,

TABLE 5. PLFA profiles of group I and II methanotrophs

PLFA	% in ^a :						
	<i>Methylomonas</i> spp. (phena 1 through 3; <i>n</i> = 4) ^b	<i>Methylococcus</i> spp. (phena 4, 5, and 8; <i>n</i> = 3) ^c	<i>Methylomonas</i> spp. (phena 6, 7, and 9; <i>n</i> = 2) ^d	<i>Methylococcus</i> <i>capsulatus</i> (phenon 10; <i>n</i> = 4) ^e	<i>Methylococcus</i> sp. (phenon 11; <i>n</i> = 2) ^f	<i>Methylococcus</i> <i>thermophilus</i> (phenon 12; <i>n</i> = 3) ^g	" <i>Methylosinus</i> " and " <i>Methylocystis</i> " spp. (phena 13 through 16; <i>n</i> = 30) ^h
i14:0	0-0.1						0-0.1
14:1ω7c	0-0.2						
14:0	18.9-24.6	7.4-9.7	0.7-1.8	0.8-6.2	1.1-1.8	1.5-1.9	0-tr
i15:0	0-2.5	0-0.4	0-7.3	0-0.1	0-5.0	0-1.3	0-0.9
a15:0	0-2.4			0-tr	0-1.0	0-0.1	0-3.5
15:1ω6c						0.4	
15:0	0-1.2	0-4.2		0-1.6	0.5	0.4-12.7	0-0.7
i16:0	0-0.2			0-0.3	0-0.6	0-0.2	0-1.4
16:1ω9c	0-tr				0-0.1		0-0.1
16:1ω8c	18.7-41.3		12.1-19.0		0-6.8		
16:1ω7c	7.7-15.3	56.8-57.4	14.1-19.9	10.6-23.1	10.4-26.3	17.2-45.9	0.3-14.2
16:1ω7t						0-2.2	0-tr
16:1ω6c	4.5-13.3	4.4-4.9	5.9-14.4	3.9-12.3	1.0-10.4	0-4.9	0-0.3
16:1ω5c	1.9-6.3	5.5-7.8	5.6-7.4	3.2-9.0	1.0-4.7	0-3.2	0-0.2
16:1ω5t	7.9-16.6	10.6-10.8	5.6-28.2	1.8-6.0	0-8.7		
16:1ω4t				0-0.4			
cy16:0						0-1.0	
16:0	4.3-8.7	8.2-8.4	11.3-18.1	33.5-56.0	22.5-22.9	39.7-47.0	0.7-5.1
i17:1ω7c	0-tr						
i17:0	0-0.1					0-0.2	0-0.3
a17:0							0-0.6
17:1ω8c				0-tr		0-0.4	0-0.2
17:1ω7c	0-0.7			0-1.8	0-1.8	0-1.1	0-0.7
17:1ω7t	0-0.3			0-2.3			
17:1ω6c							0-0.1
cy17:0	0-2.1			0-14.0	0-5.9	0-15.1	0-1.0
17:0			0-0.1	0-tr	0-0.2	0-1.4	0-0.4
cy18:0							0-6.5
18:2ω6					0-3.2		0-2.9
18:1ω13c							0-4.9
18:1ω9c	0-0.2			0-2.9	0-11.2		0-0.4
18:1ω8c							52.9-73.6
18:1ω7c	0.2-2.5	0.5-3.1	0-26.5	0-6.5	9.1-14.7	tr-3.4	14.8-37.7
18:1ω7t					0-0.5		0-4.6
18:1ω5c	0-0.2						0-0.2
18:0	0-0.1		0-2.8	0-2.1	1.6-2.7	0.1-0.7	0-5.0
br19:1	0-0.5						0-0.2
cy19:0	0.2-0.4			0.6-1.8	7.6-12.3	0-2.9	0-0.4

^a See Table 1 for strain designations. Data are from reference 5 unless otherwise specified.

^b *Methylomonas methanica* ACM 3307^T (phenon 1), "*Methylomonas rubra*" ACM 3303 (phenon 1) (this study), *Methylomonas fodinarum* ACM 3268^T (phenon 2), and *Methylomonas aurantiaca* ACM 3406^T (phenon 3).

^c *Methylococcus whittenburyi* ACM 3310^T (phenon 4), *Methylomonas* sp. strain A45 (phenon 5) (this study), and *Methylococcus luteus* ACM 3304^T (phenon 8).

^d "*Methylomona agile*" ACM 3308^T (phenon 6), "*Methylomonas alba*" ACM 3314^T (phenon 7) (this study), and *Methylomonas pelagica* ACM 3505^T (phenon 9) (this study).

^e *Methylococcus capsulatus* ACM 3302 (this study), JB115 (this study), and JB137 (this study). PLFA data for ACM 1292^T which were obtained from reference 2 are also included.

^f *Methylococcus* sp. strains JB140 and JB173.

^g *Methylococcus thermophilus* ACM 3585^T (this study), "*Methylomonas gracilis*" ACM 3315, and strain JB187 (this study).

^h "*Methylosinus trichosporium*" ACM 3311^T (phenon 13), "*Methylosinus sporium*" ACM 3306^T (phenon 14), "*Methylosinus*" sp. strain IMV-B 3006 (this study), "*Methylocystis parvus*" ACM 3309^T, "*Methylocystis minimus*" (phenon 15), and "*Methylocystis echinoides*" ACM 3495^T (phenon 16). Additional PLFA data for 24 strains isolated from a groundwater aquifer and identified as members of either the genus "*Methylosinus*" or the genus "*Methylocystis*" were obtained from reference 4.

while the genus *Methylomonas* is differentiated from other methanotrophic genera in Table 7. An extended description of the genus *Methylomonas* is given below.

Extended description of the genus *Methylomonas* (Whittenbury and Krieg 1984). Cells are rodlike to coccobacillary and 0.5 to 1.0 µm wide by 1.0 to 2.0 µm long. Motile by means of a single polar flagellum. Produces poly-β-hydroxybutyrate inclusions and desiccation-sensitive *Azotobacter* type cysts. Most strains produce capsules. Forms a surface pellicle

when grown in static liquid culture. Pink and orange carotenoid pigments are produced. Optimum growth occurs at temperatures between 25 and 35°C; no growth occurs at temperatures above 40°C. Growth occurs between pH 5.0 and 9.0; optimum growth occurs at pH 6.5 to 7.0. NaCl is not required for growth. NaCl inhibits growth at a concentration of 1.5% (wt/vol). Cells are susceptible to lysis by 0.2% (wt/vol) SDS. The Benson-Calvin cycle is not present. Urease is produced. The major PLFAs are 14:0 and 16:1ω8c.

TABLE 6. Differentiation of group I methanotrophic species

Species or phenon	Pigmentation			Growth at:		NaCl requirement	Cyst formation	Nitrate reduction	Urease activity	Phosphatase activity	Esculin hydrolysis	Utilization of:		G+C content (mol%) ^a
	Pink	Orange	Diffusible brown	Yellow	37°C	55°C						Methylamine	Trimethylamine	
Methylomonas species														
<i>M. methanica</i>	+	+	+	+	d	+	+	+	+	+	+	+	+	51-53
<i>M. fodinarum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	58-59
<i>M. aurantiaca</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	55-56
Methylobacter species														
<i>M. agilis</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	58-60
<i>M. albus</i>	+	+	+	+	d	+	+	+	+	+	+	+	+	54-56
<i>M. luteus</i>	+	+	+	+	d	+	+	+	+	+	+	+	+	49-51
<i>M. marinus</i>	+	+	+	+	d	+	+	+	+	+	+	+	+	54-55
<i>M. pelagicus</i>	+	+	+	+	d	+	+	+	+	+	+	+	+	49
<i>M. whittenburyi</i>	+	+	+	+	d	+	+	+	+	+	+	+	+	49-54
Methylococcus taxa														
<i>M. capsulatus</i>	+	+	+	+	+	d	+	+	+	+	+	d	+	62-65
Phenon 11	+	+	+	+	+	+	+	+	+	+	+	+	+	64-66
<i>M. thermophilus</i>	+	+	+	+	d	+	+	+	+	+	+	+	+	59-61

^a As determined by the *T₁* method. ^b +, 91 to 100% of the strains are positive; (+), 81 to 90% of the strains are positive; d, 21 to 81% of the strains are positive; (-), 11 to 20% of the strains are positive; -, 0 to 10% of the strains are positive.

The major ubiquinone is 18-methylene-ubiquinone 8 (only determined for *Methylomonas methanica*) (12). The G+C content is 51 to 59 mol% (*T₁*). The type species is *Methylomonas methanica* (56). Other species include *Methylomonas fodinarum* and *Methylomonas aurantiaca* (6).

Misclassified *Methylomonas* species. The species "*Methylomonas agile*," "*Methylomonas alba*," and *Methylomonas pelagica* have been compared with *Methylomonas methanica* by using only a relatively limited array of characteristics (42-45, 57). In this study the results of a numerical analysis and DNA-DNA hybridization indicated that these species are distinct from each other and from other group I methanotrophs at the species level. These species exhibit certain obvious phenotypic differences with *Methylomonas methanica*, *Methylomonas fodinarum*, and *Methylomonas aurantiaca*, including the absence of pigmentation and cyst formation. In addition, these species have different PLFA profiles; they lack significant levels of 14:0 and have a different distribution of 16:1 isomers (Table 5). The results of molecular studies, including genome characteristics (7) and the results of 5S rRNA (10) and 16S rRNA (8, 9) sequence analyses, suggest that these species are more closely related to the mesophilic, desiccation-resistant cyst-forming *Methylococcus* species. However, some ambiguities exist in these data. Genomic characteristics place "*Methylomonas agile*" ACM 3308 with the carotenoid-containing *Methylomonas* species, and this organism seems to be quite different from its close phenotypic ally "*Methylomonas alba*" (7). The similarity of "*Methylomonas agile*" and "*Methylomonas alba*" in terms of their phenotypes and PLFA profiles suggests that these organisms are generically related; recently, phylogenetic data based on 16S ribosomal DNA sequences confirmed that there is a close relationship between these species, as well as between these species and *Methylomonas pelagica* and the desiccation-resistant cyst-forming mesophilic *Methylococcus* species (9).

In the case of "*Methylomonas gracilis*" ACM 3315, DNA-DNA hybridization analysis data indicated that this name is was a synonym of *Methylococcus thermophilus*. This conclusion is also supported by the results of the numerical analysis, which placed strain ACM 3315 in phenon 12 along with *Methylococcus thermophilus*. The PLFA profile (Table 5) and the genomic characteristics (7) of "*Methylomonas gracilis*" ACM 3315 are also very similar to those of *Methylococcus thermophilus* ACM 3585^T.

***Methylococcus* species.** The results of systematic studies of methanotroph fatty acids (5, 24), protein electrophoretic patterns (21), DNA base compositions (7, 36), genome molecular weights, and DNA melting transition widths (7) clearly demonstrate the heterogeneity present in the genus *Methylococcus*. These results suggest that the genus *Methylococcus* consists of two groups of species, each of which represents a different genus.

The high-G+C-content, moderately thermophilic *Methylococcus* species make up a distinct group I methanotroph species cluster (phenon 10 through 12) (Fig. 1). This cluster includes the species *Methylococcus capsulatus* (phenon 10) and a phenotypically and genotypically allied cluster of strains forming phenon 11. Phenon 11 strains, however, possessed several phenotypic traits differentiating them from *Methylococcus capsulatus* (Table 2). Even though substantial levels of DNA homology (37% ± 8%) seem to exist between the strains of these phenon, the homology values are well below the level recommended for intraspecies relatedness. Phenon 12 includes the species *Methylococcus thermophilus*, which is phenotypically and genotypically distinct

TABLE 7. Characteristics that differentiate methanotroph genera

Characteristic	<i>Methylomonas</i>	<i>Methylobacter</i>	<i>Methylococcus</i>	<i>Methylosinus</i>	<i>Methylocystis</i>
Cell morphology					
Rods	+	D	—	—	—
Cocci or ellipsoid	—	D	+	—	+
Curved	—	—	—	D	+
Pyriform	—	—	—	D	—
Type I intracytoplasmic membranes	+	+	+	—	—
Type II intracytoplasmic membranes	—	—	—	+	+
Motility	+	D	D	+	—
Carotenoids produced	+	—	—	—	—
Cyst formation					
Desiccation sensitive	+	—	+	—	+
Desiccation resistant	—	D	—	—	—
Exospore (bud) formation	—	—	—	+	—
Lysed by 0.2% (wt/vol) SDS	+	—	—	—	—
Growth at 45°C	—	—	+	—	—
Ribulose monophosphate pathway ^b	+	+	+	—	—
Serine pathway ^b	—	—	—	+	+
Ribulose-1,5-diphosphate carboxylase	—	—	+	—	—
Nitrogen fixation	D	—	+	+	+
Major PLFA component(s)	14:0, 16:1ω8c	16:1ω7c or 16:1ω5t	16:0 and/or 16:1ω7c	18:1ω8c	18:1ω8c
Quinone ^c	MQ-8	Q-8	MQ-8	Q-8	Q-8
Proteobacterial subdivision	γ	γ	γ	α	α
G+C content (mol%) ^d	51–59	49–60	59–66	63–67	62–67

^a +, 91 to 100% of the strains are positive; D, variation among species; —, 0 to 10% of the strains are positive.

^b Predominant pathway.

^c Data from reference 12. MQ-8, 18-methylenubiquinone 8; Q-8, ubiquinone 8.

^d As determined by the *T_i* method.

from phenotypes 10 and 11. These three phenotypes exhibit high levels of similarity on the basis of genomic characteristics (7) and PLFA profiles (Table 5). As phenotypes 10 through 12 represent a distinct group separate from other group I methanotrophs, it is proposed that these strains are true members of the genus *Methylococcus*. The species include *Methylococcus capsulatus* (type species) and *Methylococcus thermophilus*. Severe problems were encountered in maintaining viable cultures of the phenotype 11 strains, and to date no representative strain is available. Thus, no species description can be proposed for phenotype 11 strains in this paper. *Methylococcus capsulatus*, *Methylococcus thermophilus*, and the phenotype 11 strains are phenotypically differentiated in Table 6, while the genus *Methylococcus* is differentiated from other methanotrophic genera in Table 7. An emended description of the genus *Methylococcus* is given below.

Emended description of the genus *Methylococcus* (Foster and Davis 1966). Cells are cocci, coccobacilli, or occasionally short rods, are 0.8 to 1.5 μm wide, 1.0 to 1.5 μm long, and occur singly or in pairs. When motile, the cells possess a single polar flagellum. Produces *Azotobacter* type cysts which do not confer desiccation resistance. Contains a partially functional Benson-Calvin cycle which allows incorporation of carbon dioxide for cellular carbon in the presence of methane, but no autotrophic growth occurs in the absence of methane. Fixes atmospheric nitrogen via an oxygen-sensitive nitrogenase. Not susceptible to lysis by 0.2% (wt/vol) SDS. Moderate thermophiles with an optimal temperature for growth between 37 and 50°C. The major PLFAs are 16:0 and 16:1ω7c. The major ubiquinone is 18-methylenubiquinone 8 (12). The G+C content is 59 to 66 mol%. The type species is *Methylococcus capsulatus*, which was originally described by Foster and Davis (20). The other species belonging to this genus is *Methylococcus thermophilus*, which was described by Malashenko et al. (35).

Misclassified *Methylococcus* species. *Methylococcus* taxa

that produce well-defined desiccation-resistant cysts (55) include the species *Methylococcus bovis*, *Methylococcus luteus*, "*Methylococcus ucrainicus*," *Methylococcus vinelandii*, and *Methylococcus whittenburyi* and the NaCl-requiring strains (33) grouped in phenotype 5. "*Methylococcus ucrainicus*," *Methylococcus vinelandii*, and *Methylococcus whittenburyi* in reality represent a single species. This conclusion is supported by the results of a numerical analysis which placed these species, as well as a strain misclassified as *Methylococcus bovis* (ACM 3504) and two unidentified strains (JB50 and JB200) (Table 1), in phenotype 4 (level of similarity [*S_J*], ≥73%). The levels of DNA homology between phenotype 4 strains ranged from 54 to 93% (Table 3). These data correlate well with the data of Lysenko et al. (34) which indicated that the species *Methylococcus chroococcus*, *Methylococcus vinelandii*, and *Methylococcus whittenburyi* exhibited DNA homology levels of 70 to 90%. The same circumstance exists for the phenotype 8 species *Methylococcus luteus* and the type strain of *Methylococcus bovis* (IMV-B 3098), which were found to exhibit a level of DNA homology of 98% and considerable phenotypic similarity (*S_J*, ≥75%). Phenotype 5 strains are phenotypically similar to phenotype 4 species but are genotypically distinct, exhibiting levels of DNA homology of only 22 to 39% with *Methylococcus whittenburyi* ACM 3310 and *Methylococcus luteus* ACM 3304^T. Species clustered in phenotypes 4, 5, and 8 have a very similar PLFA profiles (Table 5) and genome molecular weights (1.5×10^9 to 2.0×10^9) (7). The DNA base compositions of these species range from 48 to 54 mol% G+C (7, 36). The results of a phylogenetic analysis based on 5S rRNA (10) and of 16S rRNA and 16S rDNA analyses (8, 9) indicate that these species are closely related.

"*Methylomonas agile*," "*Methylomonas alba*," and *Methylomonas pelagica* were found to exhibit a substantial degree of phenotypic similarity to the desiccation-resistant, cyst-forming species in phenotypes 4, 5, and 8 (Table 2; Fig. 1).

However, because of the low number of useful phenotypic traits available to broadly classify these species, there is some difficulty in showing obvious relatedness purely on the basis of phenotypic characteristics. Although these species are genotypically distinct (Table 3), their PLFA compositions are quite similar (Table 5). The PLFA profiles of these species also differ from the profile of the desiccation-resistant, cyst-forming species, mainly in terms of 16:1 isomer distribution; however, the total contents of the various unsaturated and monounsaturated lipids (i.e., total 16:1 contents) are very similar. On the other hand, genome characteristics (7), 5S rRNA sequences (10), and 16S rRNA and ribosomal DNA sequences (8, 9) indicate that the organisms in phenon 6, 7, and 9 that do not form cysts are related to the desiccation-resistant cyst-forming *Methylococcus* species in phenon 4, 5, and 8. On the basis of this evidence it is proposed that the misclassified *Methylomonas* and *Methylococcus* species should be combined in a single genus, designated *Methylobacter* gen. nov. A similar proposal was put forward by Romanovskaya (42); however, the proposed genus "*Methylovarius*" was never validated. The members of the genus *Methylobacter* include *Methylobacter luteus* comb. nov. (the type species), *Methylobacter agilis* sp. nov., *Methylobacter albus* comb. nov., nom. rev., *Methylobacter marinus* sp. nov. (for the phenon 5 strains), *Methylobacter pelagicus* comb. nov., and *Methylobacter whittenburyi* comb. nov. These species are phenotypically differentiated from each other in Table 6, while the genus *Methylobacter* is differentiated from other methanotrophic genera in Table 7. Descriptions of *Methylobacter* gen. nov. and its species are given below.

Description of the genus *Methylobacter* gen. nov. *Methylobacter* (Me.thyl.o.bac't'er. N.L. n. *methylo*, the methyl group; Gr. n. *bacter*, rod; N.L. masc. n. *Methylobacter*, methyl rod). The gram-negative cells vary from cocci to elliptical and/or fat short rods that are 0.8 to 1.5 μm wide by 1.2 to 3.0 μm long. When motile, cells always have a single polar flagellum. If cysts are present, they appear similar to those observed in members of the genus *Azotobacter* and are desiccation resistant but not heat resistant. Benson-Calvin cycle enzymes are not present. Atmospheric nitrogen is not fixed. Cells are not lysed by 0.2% (wt/vol) SDS. Growth in liquid cultures is evenly dispersed. Carotenoid pigments are not produced. Optimal growth occurs at temperatures between 20 and 37°C; no growth occurs at temperatures over 40°C. Growth occurs between pH 5.0 and 9.0; optimum growth occurs at pH 6.5 to 7.0. The major PLFA is 16:1 ω 7c or 16:1 ω 5t, while the major ubiquinone is ubiquinone 8 (12). The G+C content varies from 49 to 60 mol%. The type species is *Methylobacter luteus*.

Description of *Methylobacter luteus* (Romanovskaya, Malashenko, and Bogachenko 1978) comb. nov. *Methylobacter luteus* (basonym, *Methylococcus luteus*; synonym, *Methylococcus bovis* Romanovskaya, Malashenko, and Bogachenko 1978). The characteristics are the same as the characteristics given in the genus description above, including the following traits. Cells are nonmotile. Forms desiccation-resistant *Azotobacter* type cysts. Does not form polyphosphate inclusions. Colonies are pale to bright yellow, irregular or round, and low convex with entire or irregular edges and have a butyrous consistency. NaCl is not required for growth. Optimal growth occurs at 30°C. Oxidase, urease, and phosphatase are produced. Does not reduce nitrate, selenite, tellurite, or triphenyltetrazolium chloride. Hydrolyzes esculin. Utilizes methane and methanol as sole sources of carbon and energy. Other traits are shown in Table 2. The G+C

content of the DNA is 49 to 51 mol% (T_i). The major PLFA component is 16:1 ω 7c. The type strain is NCIMB 11914 (= ACM 3304 = VKM-53B).

Description of *Methylobacter agilis* sp. nov. *Methylobacter agilis* (basonym, "*Methylomonas agile*" Whittenbury, Phillips, and Wilkinson 1970) (a.gi'lis. M. L. adj. *agilis*, agile). The characteristics are the same as the characteristics given in the genus description above, including the following traits. Cells are motile by means of a single polar flagellum. Does not form desiccation-resistant *Azotobacter* type cysts. Forms polyphosphate inclusions. Colonies are translucent, white, round, and low convex with entire or lobate edges and have a butyrous consistency. NaCl is not required for growth. Optimal growth occurs at 30°C. Oxidase and phosphatase are produced. Urease negative. No reduction of nitrate, selenite, or tellurite occurs. Reduces triphenyltetrazolium chloride. Esculin is hydrolyzed. Utilizes methane, methanol, and methylamine as sole sources of carbon and energy. Other traits are shown in Table 2. The G+C content of the DNA is 58 to 60 mol% (T_i). The major PLFA component is 16:1 ω 5t. The type strain is NCIMB 11124 (= A30 = ACM 3308 = ATCC 35068).

Description of *Methylobacter albus* sp. nov. *Methylobacter albus* (basonym, "*Methylomonas albus*" Whittenbury, Phillips, and Wilkinson 1970) (al'bus. M. L. adj. *albus*, white). The characteristics are the same as the characteristics given in the genus description above, including the following traits. Cells are motile by means of a single polar flagellum. Does not form desiccation-resistant *Azotobacter* type cysts. Forms polyphosphate inclusions. Colonies are white, round, and low convex with entire or lobate edges and have a butyrous consistency. NaCl is not required for growth. Optimal growth occurs at 30°C. Phosphatase is produced. Negative for oxidase, urease, and esculin hydrolysis. Reduces nitrate to nitrite but does not reduce selenite, tellurite, or triphenyltetrazolium chloride. Utilizes methane and methanol as sole sources of carbon and energy. Other traits are shown in Table 2. The G+C content of the DNA is 54 to 56 mol% (T_i). The major PLFA component is 16:1 ω 5t. The type strain is NCIMB 11123 (= VKM-BG8 = ACM 3314).

Description of *Methylobacter marinus* sp. nov. *Methylobacter marinus* (mar'i.nus. M. L. adj. *marinus*, from the sea). The characteristics are the same as the characteristics given in the genus description above, including the following traits. Cells are motile by means of a single polar flagellum. Forms desiccation-resistant *Azotobacter* type cysts. Does not form polyphosphate inclusions. Colonies are buff to brown, round, and low convex with entire edges and have a butyrous consistency. Produces a diffusible brown pigment. NaCl (approximately 100 mM) and an unknown component present in tap water are required for growth. Optimal growth occurs at 37°C. Oxidase is produced. Urease and phosphatase negative. Does not reduce nitrate, selenite, tellurite, or triphenyltetrazolium chloride. Does not hydrolyze esculin. Utilizes methane and methanol as sole sources of carbon and energy. Other traits are shown in Table 2. The G+C content of the DNA is 54 to 55 mol% (T_i). The type strain is ACM 4717. This strain, originally referred to as A4, was isolated by Lidstrom (33) from seawater near a sewage outfall in California; subsequently, the strain was methanol adapted and is now referred to as strain A45.

Description of *Methylobacter pelagicus* (Sieburth, Johnson, Eberhardt, Sieracki, Lidstrom, and Laux 1987) comb. nov. *Methylobacter pelagi*us (basonym, *Methylomonas pelagica* Sieburth, Johnson, Eberhardt, Sieracki, Lidstrom, and Laux

TABLE 8. Phenotypic characteristics that differentiate group II methanotrophic species

Characteristic	<i>Methylosinus trichosporium</i>	<i>Methylosinus sporium</i>	<i>Methylocystis parvus</i>	<i>Methylocystis echinoides</i>
Cell morphology				
Pyriform	+	—	—	—
Reniform or vibrioid	—	+	+	+
Polyphosphate or cyst formation	—	—	+	—
Spinae	—	—	—	+
Growth at 37°C	+	+	+	—
Urease activity	—	+	(+)	—
Phosphatase activity	—	+	(+)	—
Nitrogen source utilization				
L-Asparagine, L-aspartate, L-glutamine	—	+	(+)	+
L-Lysine, L-ornithine, putrescine	+	(+)	(+)	—
G+C content (mol%)	63	65–67	64–67	62

^a +, 91 to 100% of the strains are positive; (+), 81 to 90% of the strains are positive; —, 0 to 10% of the strains are positive.

1987). The characteristics are the same as the characteristics given in the genus description above, including the following traits. Cells are motile by means of a single polar flagellum. Does not form desiccation-resistant *Azotobacter* type cysts or polyphosphate inclusions. Colonies are white to buff, round, and low convex with entire edges and have a butyrous consistency. Seawater is required for growth. No growth occurs at 37°C; optimal growth occurs at 25°C. Oxidase is produced. Urease and phosphatase negative. Does not reduce nitrate, selenite, tellurite, or triphenyltetrazolium chloride. Does not hydrolyze esculin. Utilizes methane and methanol as sole sources of carbon and energy. Other traits are shown in Table 2. The G+C content of the DNA is 49 mol% (T_i). The type strain is NCIMB 2265 (= AA23).

Description of *Methylobacter whittenburyi* (Romanovskaya, Malashenko, and Bogachenko 1978) comb. nov. *Methylobacter whittenburyi* (basonym, "*Methylobacter capsulatus*" Whittenbury, Phillips, and Wilkinson 1970; synonym, *Methylococcus vinelandii* Romanovskaya, Malashenko, and Bogachenko 1978). The characteristics are the same as the characteristics given in the genus description above, including the following traits. Cells are motile by means of a single polar flagellum. Forms desiccation-resistant *Azotobacter* type cysts. Does not form polyphosphate inclusions. Colonies are round and low convex with entire edges and have a butyrous or mucoid consistency. Colonies are white; however after extended incubation colonies become brown, and a brown diffusible pigment is produced. NaCl is not required for growth. Optimal growth occurs at 30°C. Does not produce urease or phosphatase. No reduction of selenite and tellurite occurs. Esculin is hydrolyzed. Utilizes methane and methanol as sole sources of carbon and energy. The G+C content of the DNA is 49 to 54 mol% (T_i). The major PLFA component is 16:1 ω 7c. The type strain is NCIMB 11128 (= Y = ACM 3310), which replaces lectotype strain 1521, which is not extant.

Group II methanotrophs. The Group II methanotrophs comprise two groups of species (phena 13 and 14 and phena 15 and 16) which exhibit various phenotypic (Fig. 1 and Tables 2 and 8) and genotypic differences (Table 4). "*Methylosinus*" and "*Methylocystis*" strains have also been shown to differ in their protein electrophoretic patterns (21), in serology (3), and in their genome molecular weights (7). 5S rRNA sequences (10) and 16S rRNA sequences (9, 52) have also shown that "*Methylosinus*" species are distinct from but still relatively closely related to "*Methylocystis parvus*." On the other hand, the ubiquinone contents (12), DNA base

compositions, and DNA melting transition widths (7) of various group II methanotrophs have been found to be quite similar. On this basis it is proposed that *Methylosinus* gen. nov., nom. rev. and *Methylocystis* gen. nov., nom. rev. and their species should be revived from the original invalid descriptions (43, 56). Each genus has two member species, as follows: *Methylosinus trichosporium* sp. nov., nom. rev. (type species) and *Methylosinus sporium* sp. nov., nom. rev.; and *Methylocystis parvus* sp. nov., nom. rev. (type species) and *Methylocystis echinoides* sp. nov., nom. rev. The genus *Methylocystis* has potentially more member species (22); however, strains of these potential species need to be analyzed by using DNA-DNA hybridization to ascertain their distinctiveness. The group II methanotroph species are differentiated phenotypically in Table 8, while the genera are differentiated in Table 7. Descriptions of the group II methanotroph genera and species are given below.

Description of the genus *Methylosinus* gen. nov. (ex Romanovskaya, Malashenko, and Bogachenko 1978). *Methylosinus* (Me.thyl.o.si'nus. N. L. n. *methylo*, methyl group; L. n. *sinus*, bend; N. L. masc. n. *Methylosinus*, methyl bender). Gram-negative pyriform or vibrioid cells that are 0.5 to 1.0 μ m wide by 1.5 to 3.0 μ m long. Forms rosettes in logarithmic growth phase. When not present in rosettes, cells are motile by means of a polar tuft of flagella. Cells primarily reproduce by budding off heat- and desiccation-resistant exospores from the nonflagellated pole. Cells are encapsulated but do not accumulate polyphosphate. Cells contain type II intracytoplasmic membranes which are aligned parallel to the cell wall. Cells are not susceptible to lysis by SDS. Grows at temperatures between 20 and 37°C; no growth occurs at temperatures above 40°C; the optimal growth temperature is about 30°C. Grows between pH 5.5 and 9.0; the optimal pH is 6.5 to 7.0. Organic growth factors and NaCl are not required for growth. Strictly aerobic chemoheterotrophs. Group II methanotroph. Obligate utilizer of C_1 compounds via the serine transhydroxymethylase pathway. Compounds with carbon-carbon bonds do not serve as sole sources of carbon and energy. No growth occurs on complex organic media such as nutrient agar. Does not contain the Benson-Calvin cycle for CO_2 fixation but contains a complete tricarboxylic acid cycle, unlike the group I methanotrophs. Fixes atmospheric nitrogen by means of an aerotolerant nitrogenase. Produces oxidase and catalase. Unable to grow autotrophically, to denitrify, or to reduce nitrate to nitrite. Contains 18:1 ω 8c as the predominant PFLA. The major quinone is ubiquinone 8 (12). The DNA base composition

ranges from 63 to 67 mol% G+C (T_i). *Methylosinus trichosporium* is the type species.

Description of *Methylosinus trichosporium* sp. nov. (ex Romanovskaya, Malashenko, and Bogachenko, 1978). *Methylosinus trichosporium* (tri.cho.spo'ri.um. G. n. trix, hair; L. n. spora, spore; N. L. n. trichosporium, hairlike spore). The characteristics are the same as the characteristics given in the genus description above, including the following traits. Cells are pear shaped (pyriform) and 0.5 to 1.5 μ m wide by 2.0 to 3.0 μ m long. The budding of exospores from the nonflagellated ends of the cells make the cells appear somewhat like fat exclamation marks. Colonies on NMS agar are opaque, have smooth surfaces, are low convex and circular with entire edges, have a butyrous consistency, and are white to buff. No diffusible pigments are produced. Urease and phosphatase are not produced. Esculin is not hydrolyzed. Selenite and triphenyltetrazolium chloride are not reduced. Methane and methanol are the only known sole carbon and energy sources. Other traits are shown in Table 2. The G+C content of the DNA is 63 mol% (T_i). The type strain is NCIMB 11131 (= OB3b = ACM 3311 = IMET 10543 = ATCC 35070).

Description of *Methylosinus sporium* sp. nov. (ex Romanovskaya, Malashenko, and Bogachenko 1978). *Methylosinus sporium* (spo'ri.um. L. n. spora, spore; N. L. n. sporium, spore former). The characteristics are the same as the characteristics given in the genus description above, including the following traits. Cells are vibrioid to rod shaped and 0.5 to 1.0 μ m wide by 1.5 to 3.0 μ m long. Colonies on NMS agar are opaque, have smooth surfaces, are low convex and circular with entire edges, have a butyrous consistency, and are a buff to tan. A diffusible brown pigment may be produced. A bright red prodigiosine-like pigment may also occur. Urease and phosphatase are produced. Esculin is not hydrolyzed. Selenite and triphenyltetrazolium chloride are not reduced. Methane and methanol are the only known sole carbon and energy sources. The G+C content of the DNA is 65 to 67 mol% (T_i). The type strain is NCIMB 11126 (= ACM 3306 = ATCC 35069).

Description of the genus *Methylocystis* gen. nov. (ex Romanovskaya, Malashenko, and Bogachenko 1978). *Methylocystis* (Me.thyl.o.cys'tis. N. L. n. methylo, methyl group; Gr. n. cystis, bag; N. L. masc. n. *Methylocystis*, methyl bag). Gram-negative cells that are reniform or coccobacillary and 0.3 to 0.5 μ m wide by 0.5 to 1.5 μ m long. Reproduces by normal cell division. Budding division does not occur. Does not form either rosettes or exospores. May form a lipid cyst (55) which is acid fast but does not exhibit heat or desiccation resistance. Nonmotile. Encapsulated. May accumulate poly- β -hydroxybutyrate and polyphosphate. May form spinae on cell surfaces (18). Contains type II intracytoplasmic membranes which are aligned parallel to the cell wall. Grows at temperatures between 20 and 40°C; optimal growth occurs at about 30°C. Grows between pH 5.0 and 9.0; the optimal pH for growth is about 7.0. Organic growth factors and NaCl are not required for growth. Strictly aerobic chemoheterotroph. Group II methanotroph. Obligate utilizer of C_1 compounds via the serine transhydroxymethylase pathway. Compounds with carbon-carbon bonds are not used as sole sources of carbon and energy. No growth occurs on complex organic media such as nutrient agar. Does not contain the Benson-Calvin cycle for CO_2 fixation but contains a complete tricarboxylic acid cycle. Fixes atmospheric nitrogen by means of an aerotolerant nitrogenase. Produces oxidase and catalase. Unable to grow autotrophically or to denitrify. Contains 18:1 ω 8c as the predominant PLFA. The major

quinone is ubiquinone 8 (12). The DNA base composition ranges from 62 to 67 mol% G+C (T_i). *Methylocystis parvus* is the type species.

Description of *Methylocystis parvus* sp. nov. (ex Romanovskaya, Malashenko, and Bogachenko 1978). *Methylocystis parvus* (par'vus. L. masc. adj. parvus, small). The characteristics are the same as the characteristics given in the genus description above, including the following traits. Lipid cysts are formed during stationary phase, but cells do not form spinae. Polyphosphate and poly- β -hydroxybutyrate accumulate. Colonies on NMS agar are opaque, have smooth surfaces, are low convex and circular with entire edges, have a butyrous consistency, and are pale pink to tan. A brown diffusible pigment is often produced. Urease and phosphatase are produced. Esculin is not hydrolyzed. Nitrate is reduced to nitrite. Selenite and triphenyltetrazolium chloride are not reduced. Methane and methanol are the only known carbon and energy sources. The G+C content of the DNA is 64 to 67 mol% (T_i). The type strain is NCIMB 11129 (= OBBP = ACM 3309 = IMET 10483 = ATCC 35066).

Description of *Methylocystis echinoides* sp. nov. (ex Gal'chenko, Shishkina, Suzina, and Trotsenko 1977). *Methylocystis echinoides* (ech.in.oi'des. Gr. adj. echino, hedgehog-like; Gr. suff. ides, similar to; N. L. neut. adj. echinoides, spiny like a hedgehog). The characteristics are the same as the characteristics given in the genus description above, including the following traits. Cells are covered with cylindrical spinae (18). Lipid cysts are not formed. Poly- β -hydroxybutyrate and polyphosphate do not accumulate. Growth on solid NMS medium (solidified with either agar or agarose) is poor. Colonies on NMS agar are pinpoint and opaque, have smooth surfaces, are low convex and circular with entire edges, have a butyrous consistency, and are white. Diffusible pigments are not produced. Nitrate is reduced to nitrite. Urease and phosphatase are not produced. Esculin is not hydrolyzed. Selenite and triphenyltetrazolium chloride are not reduced. Methane and methanol are the only known sole carbon and energy sources. The G+C content of the DNA is 62 mol% (T_i). The type strain is IMET 10491.

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