Further Studies of the Genus *Methylobacterium* and Description of *Methylobacterium aminovorans* sp. nov.

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Pink-pigmented facultatively methylotrophs belonging to the genus *Methylobacterium* were examined in this study. *Methylobacterium* strains are divided into eight species on the basis of phenotypic characteristics. This division is supported by the results of DNA-DNA homology experiments. On the basis of DNA relatedness and other characteristics, a new species, *Methylobacterium aminovorans*, is proposed. The type strain of *M. aminovorans* is strain TH-15 (= JCM 8240).

Previously (39, 40, 43–45), we described the separation of gram-negative methanol-utilizing bacteria into 11 groups on the basis of morphological characteristics, assimilation of carbon compounds, DNA base composition, cellular fatty acid composition, ubiquinone system, and electrophoretic characteristics of enzymes. The group 2 bacteria that we described are facultatively methylotrophic, non-spore-forming, pink-pigmented, polarly flagellated (some strains have a subpolar or lateral flagellum [12]), gram-negative, rod-shaped organisms that have a ubiquinone 10 system and a cellular fatty acid composition that includes a large amount of n-C_{18:1} acid. In 1984, we (41) proposed the new genus *Protomonas* for the bacteria belonging to this group and described a single species, *Protomonas extorquens*. Furthermore, we described biovars 1, 2, and 3 of *Protomonas extorquens* on the basis of the assimilation of carbon compounds. However, this species was renamed *Methylobacterium extorquens* (Urakami and Komagata 1984) comb. nov. corrig. by Bousfield and Green (4).

More recently, we examined the bacterial biodegradation of tetramethylammonium hydroxide (TMAH) and *N,N*-dimethylformamide (DMF) by the following organisms: *Aminobacter aganoensis* TH-3 (35, 37), *Aminobacter niigataensis* DM-81 (37, 38), *Paracoccus aminophilus* DM-15 (36, 38), *Paracoccus aminovorans* DM-82 (16, 17, 36, 38), and strain TH-15^T (T = type strain) (35). Strain TH-15^T was a gram-negative, polarly flagellated, rod-shaped, pink-colony-forming bacterium. It utilized methanol, TMAH, and DMF, but not methane. It had a ubiquinone 10 system, a cellular fatty acid composition that included a large amount of n-C_{18:1} acid, and a hydroxy fatty acid composition that included a large amount of 3-hydroxy C_{14:0} acid. This bacterium should be included in the genus *Methylobacterium*, as reported previously (35).

In this paper we present a detailed recharacterization of *Methylobacterium* strains which includes phenotypic characteristics, phospholipid composition data, and DNA relatedness data and propose a new species, *Methylobacterium aminovorans*.

MATERIALS AND METHODS

Bacterial strains. The strains which we studied are shown in Table 1. Names which do not appear on the Approved Lists of Bacterial Names (31) are enclosed in quotation marks. Strain TH-15^T was isolated as a TMAH-utilizing bacterium by us (35). *Methylobacterium organophilum* TK 0047^T and “*Pseudomonas methanica*” TK 0048 were originally isolated as methane-utilizing bacteria (27, 42). A pink-pigmented facultatively methylotrophic bacterium, “*Mycoplana rubra*” TK 0002, was deposited in the National Collection of Industrial Bacteria, Aberdeen, United Kingdom, by M. R. Rhodes. All strains used in this study were maintained on medium B (41) or PYG agar (pH 7.0) (46).

Identification methods. Morphological and physiological characteristics of strain TH-15^T, *Methylobacterium organophilum* TK 0047^T, “*Pseudomonas methanica*” TK 0048, *Methylobacterium fujisawaense* TK 0065^T, *Methylobacterium radiotolerans* TK 0066 and TK 0067, and strains TK 0068 and TK 0069 were determined as described previously (41). Utilization of the following carbon compounds was determined for all bacterial strains: TMAH, DMF, *N*-methylformamide, formamide, D-fucose, L-aspartic acid, L-glutamic acid, betaine acid, sebacic acid, DL-tartaric acid, pimelic acid, suberic acid, azelaic acid, adipic acid, levulinic acid, mesaconic acid, citraconic acid, and caprylic acid. Each of these compounds was added at a concentration of 0.15% (wt/vol).

DNA base composition. DNAs were extracted by the method of Saito and Miura (30), and guanine-plus-cytosine (G+C) contents were determined by reverse-phase high-performance liquid chromatography (HPLC) as described by Tamaoka and Komagata (32).

Cellular fatty acid and hydroxy fatty acid compositions. Cellular fatty acid composition and hydroxy fatty acid composition were determined as described previously (44).

Quinone and quinone homologs. Quinone systems were determined as described previously (43). In the designations used for ubiquinones, the number indicates the number of isoprenoid units in the side chain (e.g., ubiquinone 9, ubiquinone 10, etc.).

Squalene and steroids. Squalene and steroids were determined as described previously (43).

Pigments. Carotenoid and bacteriochlorophyll pigments were detected by the method described previously (41, 43).

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TABLE 1. Bacterial strains studied

Strain	Other designation(s)	Species	Synonym	Reference(s)
TK 0001 ^T	NCIB 9399 ^T , JCM 2802 ^T	<i>Methylobacterium extorquens</i>	<i>Protomonas extorquens</i>	4, 12, 19, 41
TK 0002	NCIB 10409, JCM 2803		" <i>Mycoplana rubra</i> "	41
TK 0003	NCIB 2879, JCM 2804	<i>Methylobacterium extorquens</i>	" <i>Protaminobacter ruber</i> "	4, 12, 41
TK 0004	NCIB 9133, JCM 2805	<i>Methylobacterium extorquens</i>	<i>Pseudomonas</i> sp.	4, 12, 41
TK 0005	NCIB 9686, JCM 2806	<i>Methylobacterium extorquens</i>	<i>Pseudomonas</i> sp.	4, 12, 41
TK 0006	ATCC 21611, JCM 2807		" <i>Protaminobacter ruber</i> "	41
TK 0007	ATCC 21612, JCM 2808		" <i>Protaminobacter ruber</i> "	41
TK 0008	ATCC 21613, JCM 2809		" <i>Protaminobacter ruber</i> "	41
TK 0009	ATCC 21614, JCM 2810	<i>Methylobacterium rhodesianum</i>	" <i>Protaminobacter ruber</i> "	13, 41
TK 0010 ^T	ATCC 14821 ^T , JCM 2811 ^T	<i>Methylobacterium rhodinum</i>	<i>Pseudomonas rhodos</i>	12, 15, 41
TK 0012	NCIB 10598, JCM 2812	<i>Methylobacterium rhodesianum</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0013	NCIB 10599, JCM 2813	<i>Methylobacterium rhodesianum</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0014	NCIB 10600, JCM 2814	<i>Methylobacterium rhodesianum</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0015	NCIB 10602, JCM 2815	<i>Methylobacterium rhodesianum</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0016	NCIB 10611, JCM 2816	<i>Methylobacterium rhodesianum</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0017	NCIB 10601, JCM 2817	<i>Methylobacterium rhodesianum</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0018	NCIB 10603, JCM 2818	<i>Methylobacterium zatmanii</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0019	NCIB 10606, JCM 2819	<i>Methylobacterium zatmanii</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0020	NCIB 10607, JCM 2820	<i>Methylobacterium zatmanii</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0021	NCIB 10608, JCM 2821	<i>Methylobacterium zatmanii</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0022	NCIB 10609, JCM 2822	<i>Methylobacterium zatmanii</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0023	NCIB 10610, JCM 2823	<i>Methylobacterium zatmanii</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0024	NCIB 10612, JCM 2824	<i>Methylobacterium zatmanii</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0025	NCIB 10604, JCM 2825	<i>Methylobacterium zatmanii</i>	" <i>Pseudomonas rosea</i> "	13, 41
TK 0027 ^T	NCIB 12249 ^T	<i>Methylobacterium rhodesianum</i>	<i>Pseudomonas</i> sp.	7, 13, 28, 41
TK 0028 ^T	NCIB 12243 ^T	<i>Methylobacterium zatmanii</i>	<i>Pseudomonas</i> sp.	7, 13, 28, 41
TK 0029	ATCC 21438, JCM 2827		<i>Pseudomonas</i> sp.	41
TK 0034 ^T	ATCC 29983 ^T , JCM 2829 ^T	<i>Methylobacterium mesophilicum</i>	<i>Pseudomonas mesophila</i>	2, 12, 41
TK 0035	IAM 12099, JCM 2830	<i>Methylobacterium radiotolerans</i>	<i>Pseudomonas radiora</i>	12, 18, 41
TK 0036 ^T	IAM 12098 ^T , JCM 2831 ^T	<i>Methylobacterium radiotolerans</i>	<i>Pseudomonas radiora</i>	12, 18, 41
TK 0037	Tomomura et al. K-62		<i>Pseudomonas</i> sp.	33, 41
TK 0038	Mizoguchi et al. TK-7		" <i>Thiobacillus rubellus</i> "	25, 41
TK 0039	Kouno and Ozaki no. 617	<i>Methylobacterium fujisawaense</i>		13, 22, 41
TK 0040	ATCC 35064			6, 41
TK 0042	Urakami and Komagata BP-3			41
TK 0043	Urakami and Komagata BP-11			41
TK 0044	Urakami and Komagata BP-22			41
TK 0045	Urakami and Komagata BP-41			41
TK 0047 ^T	ATCC 27886 ^T , JCM 2833 ^T	<i>Methylobacterium organophilum</i>		27, 42
TK 0048	NRRL B-3449, JCM 2834		" <i>Pseudomonas methanica</i> "	42
TK 0065 ^T	NCIB 12417 ^T	<i>Methylobacterium fujisawaense</i>		13, 16
TK 0066	NCIB 9142	<i>Methylobacterium radiotolerans</i>	<i>Pseudomonas</i> sp.	13, 16
TK 0067	NCIB 9143	<i>Methylobacterium radiotolerans</i>	<i>Pseudomonas</i> sp.	13
TK 0068	NCIB 9141			13, 16
TK 0069	NCIB 9145			13, 16
TH-15 ^T				35

Phospholipid composition. Lipids were extracted by the procedure of Bligh and Dyer (3). Phospholipid compositions were determined by the method of Goldberg and Jensen (8).

DNA-DNA hybridization. DNA-DNA hybridization was carried out at 68°C by using the method of Kaneko et al. (20). DNAs from *Methylobacterium extorquens* TK 0001^T, *Methylobacterium organophilum* TK 0047^T, *Methylobacterium rhodinum* TK 0010^T, strain TH-15^T, and *Methylobacterium mesophilicum* TK 0034^T were labeled with [1',2',5-³H]dCTP by the nick translation method, using a type TRK 7000 kit (Amersham International plc, Amersham, United Kingdom). DNA-DNA hybridization experiments were performed with *Methylobacterium extorquens* TK 0001^T, *Methylobacterium organophilum* TK 0047^T, "*Pseudomonas methanica*" TK 0048, *Methylobacterium rhodinum* TK 0010^T, *Methylobacterium zatmanii* TK 0025, strain TH-15^T, *Methylobacterium radiotolerans* TK 0036^T, *Methylobacterium mesophilicum* TK 0034^T, and "*Thiobacillus rubellus*" TK 0038.

RESULTS

Phenotypic characteristics of pink-pigmented facultatively methylotrophic bacteria. Strain TH-15^T, *Methylobacterium organophilum* TK 0047^T, "*Pseudomonas methanica*" TK 0048, *Methylobacterium fujisawaense* TK 0065^T, *Methylobacterium radiotolerans* TK 0066 and TK 0067, and strains TK 0068 and TK 0069 were gram-negative, non-spore-forming, rod-shaped organisms. Their cells were 0.8 to 1.2 by 1.5 to 4.0 µm and had rounded ends. The cells occurred singly, rarely in pairs, and were motile by means of a single polar flagellum. Some cells had a subpolar or lateral flagellum. Granules of poly-β-hydroxybutyric acid accumulated in the cells. A pink or red water-insoluble pigment was produced, but a water-soluble fluorescent pigment was not produced by any of the strains on King B medium. Lighter pink colonies appeared frequently, and colorless colonies appeared rarely. The methyl red and Voges-Proskauer tests

were negative. Indole and hydrogen sulfide (triple sugar iron medium) were not produced. Hydrolysis of gelatin and starch was not observed. Ammonia was not produced. Acid was produced oxidatively from sugars but was not produced fermentatively. Ammonia, nitrate, and urea were assimilated as nitrogen sources. Urease, oxidase, and catalase were produced. Good growth was observed between pH 6.0 and 8.0. All bacteria tested grew at 30°C. Growth did not occur in the presence of 3% sodium chloride. These characteristics were identical to those of other *Methylobacterium* strains (41).

All of the strains used in this study grew at the expense of methanol, glycerol, and succinic acid, but did not grow at the expense of methane, maltose, sucrose, lactose, sorbitol, D-mannitol, inositol, or soluble starch. However, utilization of L-arabinose, D-xylose, D-glucose, mannose, D-fructose, galactose, trehalose, citric acid, ethanol, monomethylamine, dimethylamine, trimethylamine, TMAH, DMF, formamide, N-methylformamide, D-fucose, L-aspartic acid, L-glutamic acid, betaine, sebamic acid, DL-tartaric acid, pimelic acid, suberic acid, azelaic acid, and adipic acid differed among the strains (Table 2). Only strain TH-15^T utilized TMAH, formamide, N-methylformamide, and DMF. Oxidative production of acid from sugars was variable among the strains (Table 3). Vitamins and amino acids were not essential for the growth of all strains, and calcium pantothenate stimulated the growth of almost one-half of the strains (Table 3).

DNA base compositions. The DNA base compositions of *Methylobacterium extorquens* TK 0001^T, "*Mycoplana rubra*" TK 0002, *Methylobacterium organophilum* TK 0047^T, "*Pseudomonas methanica*" TK 0048, *Methylobacterium rhodinum* TK 0010^T, *Methylobacterium rhodesianum* TK 0027^T, "*Pseudomonas rosea*" TK 0012, *Methylobacterium zatmanii* TK 0028^T and TK 0025, strain TH-15^T, *Methylobacterium radiotolerans* TK 0036^T and TK 0035, *Methylobacterium mesophilum* TK 0034^T, *Methylobacterium fujisawaense* TK 0065^T, and "*T. rubellus*" TK 0038 ranged from 68 to 72 mol% G+C (Table 4).

Cellular fatty acid compositions. *Methylobacterium fujisawaense* TK 0065^T, *Methylobacterium radiotolerans* TK 0066 and TK 0067, and strains TK 0068 and TK 0069 contained a large amount of C_{18:1} acid and small amounts of C_{16:1} acid, C_{19:0} cyclopropane acid, and 3-OH C_{14:0} hydroxy acid. The hydroxy fatty acids included a large amount of 3-hydroxy C_{14:0} acid. These cellular fatty acid compositions were as the same as the cellular fatty acid compositions of other *Methylobacterium* strains (35, 41, 44).

Quinone systems. Like other *Methylobacterium* strains (35, 41, 43), *Methylobacterium fujisawaense* TK 0065^T, *Methylobacterium radiotolerans* TK 0066 and TK 0067, and strains TK 0068 and TK 0069 had a ubiquinone 10 system, as well as minor ubiquinone 8, ubiquinone 9, and ubiquinone 11 components.

Squalene and steroids. Like other *Methylobacterium* strains (41, 43), strain TH-15^T, *Methylobacterium fujisawaense* TK 0065^T, *Methylobacterium radiotolerans* TK 0066 and TK 0067, and strains TK 0068 and TK 0069 contained hop-22 (29)-ene and hopan-22-ol and a small amount of squalene.

Pigments. Like other *Methylobacterium* strains (41, 43), strain TH-15^T, *Methylobacterium fujisawaense* TK 0065^T, *Methylobacterium radiotolerans* TK 0066 and TK 0067, and strains TK 0068 and TK 0069 contained carotenoid pigments which had absorption maxima at 465, 495, and 525 nm and bacteriochlorophyll *a*.

Phospholipid compositions. The phospholipids of all strains

included large amounts of cardiolipin (diphosphatidylglycerol), phosphatidylethanolamine, and phosphatidylcholine and a small amount of phosphatidylglycerol (Fig. 1).

DNA-DNA hybridization. The levels of DNA relatedness among *Methylobacterium* strains are shown in Table 5. The strains tested were divided into seven groups (groups A through G), and the similarity values between groups were less than 60%. *Methylobacterium extorquens* TK 0001^T (group A) was clearly distinguished from other *Methylobacterium* strains examined in this study. *Methylobacterium organophilum* TK 0047^T (group B) and "*Pseudomonas methanica*" TK 0048 exhibited high level (73%) of similarity with each other and were clearly separated from other *Methylobacterium* strains. *Methylobacterium mesophilum* TK 0034^T (group G) and "*T. rubellus*" TK 0038 exhibited a high level (68%) of similarity with each other and were clearly separated from other *Methylobacterium* strains.

DISCUSSION

"*Mycoplana rubra*" TK 0002, "*Protaminobacter ruber* subsp. *machidanus*" TK 0006, TK 0007, and TK 0008, *Pseudomonas* sp. strains TK 0029 and TK 0037 (= Tonomura et al. K-62), "*T. rubellus*" TK 0038, strains TK 0040 (= ATCC 35064), TK 0042, TK 0043, TK 0044, TK 0045, TK 0068 (= NCIB 9141), TK 0069 (= NCIB 9145), and TH-15^T, and "*Pseudomonas methanica*" TK 0067 should be included in the genus *Methylobacterium* on the basis of their morphological characteristics and their chemotaxonomic characteristics, including their cellular fatty acid compositions (35, 41, 44), their hydroxy fatty acid compositions (35, 41, 44), their quinone systems (35, 41, 43), their DNA base compositions (41, 45), the presence of carotenoid pigments (41), bacteriochlorophyll *a* (41), squalene, and steroids [hop-22(29)-ene and hopan-22-ol] (41, 43), their phospholipid compositions, and the levels of DNA-DNA homology (16). Goldberg and Jensen (8) reported that the phospholipids of pink-pigmented, facultatively methylotrophic bacteria included large amounts of phosphatidylethanolamine, phosphatidylcholine, and phosphatidylglycerol and a small amount of phosphatidylglycerol. The results of these authors differed from our results in the ratio of phosphatidylglycerol to diphosphatidylglycerol. The reason for this unexpected observation is not apparent.

In 1976, the genus *Methylobacterium* was proposed as a new genus of rod-shaped, methane-oxidizing, facultatively methylotrophic bacteria by Patt et al. (27). This genus was established on the basis of only one strain, *Methylobacterium organophilum* XX^T (= ATCC 27886^T), and this strain was markedly different from the members of other methane-oxidizing bacterial genera, such as the genera *Methylomonas*, *Methylococcus*, "*Methylosinus*," and "*Methylovibrio*" (47), in its ability to utilize a variety of organic substrates with carbon-carbon bonds as sources of carbon and energy. Subsequently, *Methylobacterium organophilum* R6 (26) and CRL-26 (17), "*Methylobacterium ethanolicum*" (24), and "*Methylobacterium hypolimneticum*" (24) were described as facultatively methane-utilizing bacteria. However, lack of utilization of methane by *Methylobacterium organophilum* ATCC 27886^T (= TK 0047^T) was reported by Green and Bousfield (12), Romanovskaya et al. (29), and Urakami and Komagata (42). On the other hand, Hanson (14) suggested that a plasmid plays a role in the utilization of methane by *Methylobacterium organophilum*. Lidstrom-O'Connor et al. (23) isolated two methylotrophic bacteria, an obligately methanotrophic bacterium and a *Xanthobacter* species, from

TABLE 2. Assimilation of carbon compounds by *Methylobacterium* strains

Strain	Species	Utilization of:																							
		L- Arabinose	D- Xylose	D- Glucose	Mannose	D- Fructose	Galactose	Trehalose	Citric acid	Ethanol	Mono-methylamine	Di-methylamine	Tri-methylamine	TMAH	DMF	D- Fucose	L- Aspartic acid	L- Glutamic acid	Betaine	Sebacic acid	DL-Tartaric acid	Pimelic acid	Suberic acid	Azelaic acid	Adipic acid
TK 0001 ^T	<i>Methylobacterium extorquens</i>	-	-	-	-	-	-	-	-	+	w ^a	-	-	-	-	-	+	+	+	-	-	-	-	-	-
TK 0002		-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	w	+	-	-	-	-	-	-
TK 0003	<i>Methylobacterium extorquens</i>	-	-	-	-	-	-	-	-	+	w	-	-	-	-	-	+	+	+	-	-	-	-	-	-
TK 0004	<i>Methylobacterium extorquens</i>	-	-	-	-	-	-	-	-	+	w	-	-	-	-	-	+	+	+	-	-	-	-	-	-
TK 0005	<i>Methylobacterium extorquens</i>	-	-	-	-	-	-	-	-	+	w	-	-	-	-	-	+	+	+	-	-	-	-	-	-
TK 0047 ^T	<i>Methylobacterium organophilum</i>	-	-	-	-	+	-	-	-	+	w	-	+	-	-	-	w	w	-	-	-	-	-	-	-
TK 0048		-	-	-	-	+	-	-	-	+	w	-	+	-	-	-	+	w	w	-	-	-	-	-	-
TK 0010 ^T	<i>Methylobacterium rhodinum</i>	-	-	-	-	+	-	-	+	+	w	-	-	-	-	-	+	w	+	-	w	-	-	-	-
TK 0069		-	-	-	-	+	-	-	+	w	w	-	-	-	-	-	w	w	+	-	w	-	-	-	-
TK 0027 ^T	<i>Methylobacterium rhodesianum</i>	-	-	-	-	+	-	-	-	w	+	+	-	-	-	-	w	w	w	-	w	-	-	-	-
TK 0006		-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	+	+	-	w	-	-	-	-
TK 0007		-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	+	+	-	w	-	-	-	-
TK 0008		-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	w	-	-	-	-	-	-	-
TK 0009	<i>Methylobacterium rhodesianum</i>	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	w	+	-	-	-	-	-	-
TK 0012	<i>Methylobacterium rhodesianum</i>	-	-	-	-	+	-	-	-	+	w	+	-	-	-	-	+	+	+	-	-	-	-	-	-
TK 0013	<i>Methylobacterium rhodesianum</i>	-	-	-	-	+	-	-	-	+	w	+	-	-	-	-	+	+	+	-	-	-	-	-	-
TK 0014	<i>Methylobacterium rhodesianum</i>	-	-	-	-	+	-	-	-	+	w	+	-	-	-	-	+	+	+	-	-	-	-	-	-
TK 0015	<i>Methylobacterium rhodesianum</i>	-	-	-	-	+	-	-	-	+	w	+	-	-	-	-	+	+	+	-	-	-	-	-	-
TK 0016	<i>Methylobacterium rhodesianum</i>	-	-	-	-	+	-	-	-	+	w	+	-	-	-	-	+	+	+	-	-	-	-	-	-
TK 0017	<i>Methylobacterium rhodesianum</i>	-	-	-	-	+	-	-	-	+	w	+	-	-	-	-	+	+	+	-	-	-	-	-	-
TK 0029		-	-	-	-	+	-	-	-	+	w	-	-	-	-	-	+	+	+	-	w	-	-	-	-
TK 0068		-	-	-	-	+	-	-	-	w	+	-	-	-	-	-	w	w	+	-	w	-	-	-	-
TK 0028 ^T	<i>Methylobacterium zatmanii</i>	-	-	-	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	w	-	-	-	-
TK 0018	<i>Methylobacterium zatmanii</i>	-	-	-	-	+	-	-	-	+	w	-	+	-	-	-	-	-	-	-	w	-	-	-	-
TK 0019	<i>Methylobacterium zatmanii</i>	-	-	-	-	+	-	-	-	+	w	-	+	-	-	-	-	-	-	-	w	-	-	-	-
TK 0020	<i>Methylobacterium zatmanii</i>	-	-	-	-	+	-	-	-	+	w	-	+	-	-	-	-	-	-	-	w	-	-	-	-
TK 0021	<i>Methylobacterium zatmanii</i>	-	-	-	-	+	-	-	-	+	w	-	+	-	-	-	-	-	-	-	w	-	-	-	-
TK 0022	<i>Methylobacterium zatmanii</i>	-	-	-	-	+	-	-	-	+	w	-	+	-	-	-	-	-	-	-	w	-	-	-	-
TK 0023	<i>Methylobacterium zatmanii</i>	-	-	-	-	+	-	-	-	+	w	-	+	-	-	-	-	-	-	-	w	-	-	-	-
TK 0024	<i>Methylobacterium zatmanii</i>	-	-	-	-	+	-	-	-	+	w	-	+	-	-	-	-	-	-	-	w	-	-	-	-
TK 0025	<i>Methylobacterium zatmanii</i>	-	-	-	-	+	+	-	-	+	w	-	+	-	-	-	-	-	-	-	w	-	-	-	-
TH-15 ^T		-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	w	+	-	w	-	-	-	-
TK 0036 ^T	<i>Methylobacterium radiotolerans</i>	+	+	+	-	-	+	-	+	w	-	-	-	-	-	+	w	+	+	+	w	+	+	+	w
TK 0035	<i>Methylobacterium radiotolerans</i>	+	w	+	-	-	+	-	+	w	-	-	-	-	-	+	+	+	+	+	w	+	+	+	w
TK 0066	<i>Methylobacterium radiotolerans</i>	+	+	+	-	-	w	-	+	-	-	-	-	-	-	+	w	+	+	+	w	+	+	+	w
TK 0067	<i>Methylobacterium radiotolerans</i>	+	+	+	-	-	+	-	+	-	-	-	-	-	-	+	w	+	+	+	w	+	+	+	w
TK 0034 ^T	<i>Methylobacterium mesophilicum</i>	+	w	+	-	-	+	-	+	w	-	-	-	-	-	w	w	+	-	+	-	w	+	w	w
TK 0065 ^T	<i>Methylobacterium fujisawaense</i>	+	+	+	-	+	-	+	+	+	-	-	-	-	-	+	w	+	-	+	-	+	+	+	w
TK 0039	<i>Methylobacterium fujisawaense</i>	w	w	+	-	-	+	-	+	w	-	-	-	-	-	+	+	+	-	+	-	+	+	+	+
TK 0042		w	w	+	w	-	+	-	+	-	-	-	-	-	-	+	+	+	-	+	-	+	+	+	+
TK 0043		w	+	+	-	-	+	w	w	w	-	-	-	-	-	+	+	+	-	+	w	+	+	+	w
TK 0038		+	w	w	-	+	+	-	w	w	-	-	-	-	-	+	-	+	-	w	-	w	+	+	w
TK 0037		+	+	+	-	+	+	w	+	-	-	-	-	-	-	+	-	+	-	+	-	+	+	+	w
TK 0040		+	+	+	-	+	+	-	+	w	-	-	-	-	-	+	+	+	+	+	-	+	+	+	w
TK 0044		w	+	+	-	+	+	-	+	-	-	-	-	-	-	+	w	+	-	+	-	+	+	+	+
TK 0045		w	+	+	w	w	+	-	+	-	-	-	-	-	-	+	w	+	-	+	-	+	+	+	+

^a w, weak.

TABLE 3. Oxidative production of acid from sugars and vitamin requirements of *Methylobacterium* strains

Strain	Species	Oxidation of:						Requirement for calcium pantothenate as a stimulate
		L- Arabinose	D- Arabinose	D- Glucose	D- Fructose	Galactose	Glycerol	
TK 0001 ^T	<i>Methylobacterium extorquens</i>	w ^a	w	-	-	-	+	w
TK 0002		w	w	-	-	-	+	w
TK 0003	<i>Methylobacterium extorquens</i>	w	w	-	-	-	+	w
TK 0004	<i>Methylobacterium extorquens</i>	w	w	-	-	-	+	w
TK 0005	<i>Methylobacterium extorquens</i>	w	w	-	-	-	+	w
TK 0047 ^T	<i>Methylobacterium organophilum</i>	w	w	w	w	-	+	w
TK 0048		w	w	w	w	-	+	w
TK 0010 ^T	<i>Methylobacterium rhodinum</i>	w	w	-	-	-	+	w
TK 0069		w	w	-	w	-	+	w
TK 0027 ^T	<i>Methylobacterium rhodesianum</i>	w	w	-	+	-	+	w
TK 0006		w	+	-	w	-	+	w
TK 0007		w	w	-	w	-	+	w
TK 0008		w	+	-	w	-	+	w
TK 0009	<i>Methylobacterium rhodesianum</i>	w	+	-	w	-	+	w
TK 0012	<i>Methylobacterium rhodesianum</i>	w	+	-	w	-	+	w
TK 0013	<i>Methylobacterium rhodesianum</i>	w	+	-	w	-	+	w
TK 0014	<i>Methylobacterium rhodesianum</i>	w	w	-	w	-	+	w
TK 0015	<i>Methylobacterium rhodesianum</i>	w	+	-	w	-	+	w
TK 0016	<i>Methylobacterium rhodesianum</i>	w	w	-	w	-	+	w
TK 0017	<i>Methylobacterium rhodesianum</i>	w	w	-	w	-	+	w
TK 0029		w	w	-	w	-	w	w
TK 0068		w	+	-	w	-	+	w
TK 0028 ^T	<i>Methylobacterium zatmanii</i>	w	+	-	w	-	+	w
TK 0018	<i>Methylobacterium zatmanii</i>	w	+	-	w	-	+	w
TK 0019	<i>Methylobacterium zatmanii</i>	w	+	-	w	-	+	w
TK 0020	<i>Methylobacterium zatmanii</i>	w	+	-	w	-	+	w
TK 0021	<i>Methylobacterium zatmanii</i>	w	+	-	w	-	+	w
TK 0022	<i>Methylobacterium zatmanii</i>	w	+	-	w	-	+	w
TK 0023	<i>Methylobacterium zatmanii</i>	w	+	-	w	-	+	w
TK 0024	<i>Methylobacterium zatmanii</i>	w	+	-	w	-	+	w
TK 0025	<i>Methylobacterium zatmanii</i>	w	+	-	w	-	+	w
TH-15 ^T		w	w	-	w	-	+	w
TK 0036 ^T	<i>Methylobacterium radiotolerans</i>	-	-	-	-	-	-	+
TK 0035	<i>Methylobacterium radiotolerans</i>	-	-	-	-	-	+	w
TK 0066	<i>Methylobacterium radiotolerans</i>	+	w	-	-	+	+	w
TK 0067	<i>Methylobacterium radiotolerans</i>	+	-	-	-	+	-	w
TK 0034 ^T	<i>Methylobacterium mesophilicum</i>	-	w	-	-	-	-	+
TK 0065 ^T	<i>Methylobacterium fujisawaense</i>	w	w	-	-	-	-	+
TK 0039	<i>Methylobacterium fujisawaense</i>	+	+	-	-	+	+	+
TK 0042		+	+	-	-	-	+	w
TK 0043		-	+	-	-	-	-	+
TK 0038		-	+	-	-	-	+	w
TK 0037		+	+	-	w	+	w	+
TK 0040		w	w	-	w	-	+	+
TK 0044		+	+	-	-	+	-	w
TK 0045		+	+	-	-	+	-	w

^a w, weak.

cultures of "*Methylobacterium ethanolicum*" which were grown in methane. Therefore, at present the existence of facultatively methanotrophic bacteria is questionable.

In 1983, Green and Bousfield (12) proposed emendation of the genus *Methylobacterium* description to permit inclusion of pink-pigmented, non-methane-utilizing, facultatively methylotrophic bacteria and described four species, *Methylobacterium organophilum*, *Methylobacterium rhodinum*, *Methylobacterium radiotolerans*, and *Methylobacterium mesophilicum*. Furthermore, in 1985 Bousfield and Green (4) proposed reclassification of the genus *Protomonas* Urakami and Komagata 1984 and transfer of *Protomonas extorquens* to the genus *Methylobacterium* (Patt, Cole, and Hanson)

emend. Green and Bousfield 1983. *Protomonas extorquens* was reclassified as *Methylobacterium extorquens*. Although the utilization of methane by *Methylobacterium* strains was not confirmed by these authors, their emended description of the genus *Methylobacterium* did not exclude methane-utilizing bacteria (12). The utilization of methane is a very important characteristics of the genus *Methylobacterium* as established by Patt et al. (27); therefore, nonutilization of methane should be mentioned in the description of the genus *Methylobacterium*. Furthermore, DNA base compositions ranging from 60 to 70 mol% G+C show that the genus is heterogeneous. Hood et al. (16) studied the DNA base compositions of *Methylobacterium* strains by using the

TABLE 4. DNA base compositions of *Methylobacterium* strains

Strain	Species	G+C content (mol%)			
		This study ^a	Study of Hood et al. ^b		Other studies ^c
			Buoyant density method	Thermal denaturation method	
TK 0001 ^T	<i>Methylobacterium extorquens</i>	68.2	69.4	69.1	65.6 (<i>T_m</i>)
TK 0002		68.3			
TK 0003	<i>Methylobacterium organophilum</i>	68.8	71.1	70.5	65.6 (<i>T_m</i>)
TK 0047 ^T		69.2			
TK 0048	<i>Methylobacterium rhodinum</i>	70.5	71.8		66.8 (<i>T_m</i>), 66.0 (BD)
TK 0010 ^T		69.2			
TK 0027 ^T	<i>Methylobacterium rhodesianum</i>	70.2			66.0 (<i>T_m</i>)
TK 0012		68.9			
TK 0016	<i>Methylobacterium zatmanii</i>	69.2	70.9		65.4 (<i>T_m</i>)
TK 0028 ^T		68.8			
TK 0025	<i>Methylobacterium radiotolerans</i>	69.3	70.3		66.1 (<i>T_m</i>)
TH-15 ^T		68.0			
TK 0036 ^T	<i>Methylobacterium mesophilicum</i>	71.2	72.3		65.9 (<i>T_m</i>)
TK 0035		70.9			
TK 0034 ^T	<i>Methylobacterium fujisawaense</i>	69.5	69.9		66.0 (<i>T_m</i>), 65.8 (<i>T_m</i>)
TK 0065 ^T		69.9			
TK 0038		69.5			65.0, 65.4 (<i>T_m</i>)

^a G+C contents were determined by reverse-phase HPLC.^b Data from reference 16.^c Data from references 2, 18, 25, 26, and 45. The values were determined by the thermal denaturation method (*T_m*) or the buoyant density method (BD).

buoyant density and thermal denaturation methods, but their values were approximately 5 mol% higher than the values determined by other researchers (2, 18, 25, 27, 45), as shown in Table 4. In this study, the DNA base compositions determined by the HPLC method nearly coincided with the values determined by Hood et al. As determined in this study, the DNA base compositions of the genus *Methylobacterium* strains are 68 to 72 mol%. In the recently published second edition of *The Prokaryotes* (10), Green states that the DNA base compositions of members of the genus *Methylobacterium* range from 68.4 to 72.4 mol% G+C.

Recently, a genotypic study of *Methylobacterium* strains was carried out. Wolfrum et al. (48) reported the levels of nucleic acid hybridization for bacteria belonging to the genera *Methylobacterium* and *Pseudomonas*. Furthermore, the 5S rRNA sequences of *Methylobacterium extorquens* TK 0001^T (21), *Methylobacterium extorquens* TK 0004 (6), and *Methylobacterium organophilum* TK 0047^T (6) were determined, and the phylogenetic diversity of methanol-utilizing bacteria was discussed by Ando et al. (1). The 16S rRNA sequences of methylotrophic bacteria, including *Methylobacterium extorquens* and *Methylobacterium organophilum* (5, 34), have been determined. On the basis of these studies, *Methylobacterium* strains were clearly distinguished from other methylotrophic bacteria (strains belonging to the genera *Methylobacillus*, *Methylophilus*, *Hyphomicrobium*, *Ancylobacter*, and *Paracoccus*), methanotrophic bacteria (strains belonging to the genera *Methylomonas* and *Methylococcus*), and *Pseudomonas* strains.

Methylobacterium strains were divided into eight species on the basis of utilization of carbon compounds, and this grouping was supported by electrophoretic enzyme type (40) and DNA-DNA homology data (Table 5). Group 1 contained *Methylobacterium extorquens*, group 2 contained *Methylobacterium organophilum*, group 3 contained *Methylobacterium rhodinum*, group 4 contained *Methylobacterium rhodesianum*, group 5 contained *Methylobacterium zatmanii*, group 6 contained strain TH-15^T, and group 7 contained

Methylobacterium radiotolerans. Group 8 contained *Methylobacterium fujisawaense*, *Methylobacterium mesophilicum*, *Pseudomonas* sp. strain TK 0037, "*T. rubellus*" TK 0038, and strains TK 0040 (= ATCC 35064), TK 0042, TK 0043, TK 0044, and TK 0045. *Methylobacterium mesophilicum* and *Methylobacterium fujisawaense*, which were distinguished from each other on the basis of DNA-DNA homology data (15) (Table 6), were not distinguished from each other on the basis of phenotypic characteristics. Green et al. (13) have described differences between *Methylobacterium mesophilicum* and *Methylobacterium fujisawaense* in the ability to utilize D-fructose and acetate as sole carbon sources and in the ability to grow on peptone-rich media. However, the difference between these two species was not confirmed in this study. Therefore, strains TK 0037, TK 0038, TK 0040, TK 0042, TK 0043, and TK 0044 were not identified in this study. From the data which we obtained and from practical considerations of identification, we believe that these two species should be characterized more precisely in the future insofar as this is possible.

The minimal characteristics for differentiating species in the genus *Methylobacterium* are shown in Table 6. However, the utilization of D-glucose by *Methylobacterium rhodinum* and *Methylobacterium organophilum* differs from the results of Green (10) and Green et al. (13). The reason for this unexpected observation is not apparent. The levels of DNA-DNA homology between strain TH-15^T and the other *Methylobacterium* strains examined (28 to 56%) suggest that strain TH-15^T may represent a new species. In addition, strain TH-15^T was the only organism examined in this study which could utilize TMAH and DMF as sole carbon sources (Table 6). Therefore, we believe that strain TH-15^T should be separated from other methylotrophic bacteria at the species level; consequently, we propose a new species, *Methylobacterium aminovorans*, for this strain. The differential characteristics of *Methylobacterium* species are shown in Table 7.

On the basis of our data and the data in a previous report (41), "*Pseudomonas methanica*" TK 0048 (= NRRL

FIG. 1. Thin-layer chromatographic patterns of lipids of *Methylobacterium* strains. CL, cardiolipin; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine.

B-3449) should be identified as a strain of *Methylobacterium organophilum*; “*Mycoplana rubra*” TK 0002, “*Protaminobacter ruber*” TK 0003, *Pseudomonas* sp. strain TK 0004 (= AM 1), and *Pseudomonas* sp. strain TK 0005 (= M27), which were included in *Protomonas extorquens* biovar 1 (41), should be identified as strains of *Methylobacterium extorquens*; strain TK 0069 should be identified as a strain of *Methylobacterium rhodinum*; “*Protaminobacter ruber* subsp. *machidanus*” TK 0006, TK 0007, TK 0008, and TK 0009, “*Pseudomonas rosea*” TK 0012, TK 0013, TK 0014, TK 0015, TK 0016, and TK 0017, *Pseudomonas* sp. strain TK 0029, and strain TK 0068 should be identified as strains of *Methylobacterium rhodesianum*; and “*Pseudomonas rosea*” TK 0018, TK 0019, TK 0020, TK 0021, TK 0022, TK 0023, TK 0024, and TK 0025 should be included in *Methylobacterium zatmanii*. The taxonomic positions of *Pseudomonas* sp. strain TK 0037, “*T. rubellus*” TK 0038, and strains TK 0040 (= ATCC 35064), TK 0042, TK 0043, TK 0044, and TK 0045 were not determined in this study, but these bacteria may be members of *Methylobacterium mesophilicum* or *Methylobacterium fujisawaense*.

Description of *Methylobacterium aminovorans* Urakami, Araki, Suzuki, and Komagata sp. nov. *Methylobacterium aminovorans* (a.mi.no'vo.rans. N.L.n *aminus*, amine; L. part.adj. *vorans*, devouring, digesting; N.L.part.adj. *aminovorans*, amine digesting) cells are non-spore-forming, gram-negative rods that are 0.8 to 1.0 by 1.5 to 4.0 μ m and have rounded ends. Cells occur singly or rarely in pairs. Cells are motile by means of a single polar flagellum. Some cells have a subpolar or lateral flagellum. Granules of poly- β -hydroxybutyric acid accumulate in the cells. Colonies on glucose-yeast extract-peptone agar are shiny, smooth, raised, entire, pink or red, and 1 to 3 mm in diameter after 5 days at 30°C. Lighter pink colonies appear frequently, and colorless colonies appear rarely. The red or pink pigment is a carotenoid pigment, and absorption maxima occur at 465, 495, and 525 nm. Bacteriochlorophyll *a* is formed under specific culture conditions.

Nitrate is reduced to nitrite. Methyl red and Voges-Proskauer tests are negative. Indole and hydrogen sulfide are not produced. Hydrolysis of gelatin and starch is not observed. Ammonia is not produced. Acid is produced oxida-

TABLE 5. Levels of DNA-DNA homology of *Methylobacterium* strains

Strain	Species	% DNA-DNA homology with strain:					DNA-DNA homology group
		TK 0001 ^T	TK 0047 ^T	TK 0010 ^T	TH-15 ^T	TK 0034 ^T	
TK 0001 ^T	<i>Methylobacterium extorquens</i>	100	23	45	40	30	A
TK 0047 ^T	<i>Methylobacterium organophilum</i>	31	100	36	32	36	B
TK 0048		27	73	48	36	36	B
TK 0010 ^T	<i>Methylobacterium rhodinum</i>	18	30	100	53	18	C
TK 0025	<i>Methylobacterium zatmanii</i>	46	33	57	56	49	D
TH-15 ^T		47	30	55	100	43	E
TK 0036 ^T	<i>Methylobacterium radiotolerans</i>	24	24	39	31	55	F
TK 0034 ^T	<i>Methylobacterium mesophilicum</i>	17	17	12	30	100	G
TK 0038		24	26	40	28	68	G

TABLE 6. Grouping of *Methylobacterium* strains based on assimilation type, electrophoretic type, and DNA-DNA homology type

Assimilation group	Species	Strain(s)	Electrophoretic type (subgroup) ^a	DNA-DNA homology group	
				This study	Study of Hood et al. ^b
1	<i>Methylobacterium extorquens</i>	TK 0001 ^T	1	A	1B
		TK 0002-TK 0005	1		
2	<i>Methylobacterium organophilum</i>	TK 0047 ^T		B	1H
		TK 0048		B	
3	<i>Methylobacterium rhodinum</i>	TK 0010 ^T		C	1G
		TK 0069			
4	<i>Methylobacterium rhodesianum</i>	TK 0027 ^T	3		1C
		TK 0006-TK 0009, TK 0012-TK 0017, TK 0029	3		
		TK 0068			
5	<i>Methylobacterium zatmanii</i>	TK 0028 ^T	2		1A
		TK 0025	2	D	
		TK 0018-TK 0024	2		
6		TH-15 ^T		E	
7	<i>Methylobacterium radiotolerans</i>	TK 0036 ^T	4	F	2B
		TK 0035	4		
		TK 0066			2B
		TK 0067			
8	<i>Methylobacterium mesophilicum</i>	TK 0034 ^T	4	G	2E
	<i>Methylobacterium fujisawaense</i>	TK 0065 ^T			2D
	<i>Methylobacterium</i> sp.	TK 0042, TK 0043	4		
	<i>Methylobacterium</i> sp.	TK 0038	4	G	
		TK 0037, TK 0040, TK 0044, TK 0045	4		

^a Data from reference 40.^b Data from reference 16.

tively from glycerol, but is not produced from D-glucose or galactose. Methanol, D-fructose, glycerol, ethanol, mono-methylamine, dimethylamine, trimethylamine, TMAH, formamide, N-methylformamide, DMF, succinic acid, L-aspartic acid, L-glutamic acid, and betaine are utilized, but L-arabinose, D-xylose, D-glucose, mannose, galactose, trehalose, maltose, sucrose, lactose, D-sorbitol, D-mannitol, inositol, soluble starch, citric acid, D-fucose, sebacic acid, DL-tartaric acid, pimelic acid, suberic acid, azelaic acid, adipic acid, hydrogen, and methane are not utilized. Growth factors are not required. Ammonia, nitrate, and urea are assimilated as nitrogen sources. Urease, oxidase, and cata-

lase are produced. Aerobic. Metabolism is strictly respiratory and not fermentative.

Good growth occurs between pH 6.0 and 8.0 and at 30°C. Growth does not occur in the presence of 3% sodium chloride. The DNA base composition is 68.0 mol% G+C. The cellular fatty acids include a large amount of octadecenoic (C_{18:1}) acid. The major hydroxy fatty acid is 3-hydroxy C_{14:0} acid. The phospholipids include large amounts of cardiolipin, phosphatidylethanolamine, and phosphatidylcholine. The ubiquinone system is ubiquinone 10. Methanol is utilized by the serine pathway with formaldehyde incor-

TABLE 7. Differential characteristics of *Methylobacterium* species

Species or strain(s)	Utilization of:																		Requirement for calcium pantothenate as a stimulate	
	L-Arabinose	D-Xylose	D-Glucose	D-Fructose	Galactose	Citric acid	Mono-methylamine	Di-methylamine	Tri-methylamine	TMAH	DMF	D-Fucose	L-Glutamic acid	Betaine	Sebacic acid	Pimelic acid	Suberic acid	Azelaic acid		Adipic acid
<i>Methylobacterium extorquens</i>	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-
<i>Methylobacterium organophilum</i>	-	-	-	+	-	-	+	-	+	-	-	-	+	-/w	-	-	-	-	-	-
<i>Methylobacterium rhodinum</i>	-	-	-	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-
<i>Methylobacterium rhodesianum</i>	-	-	-	+	-	-	+	+/-	-	-	-	-	+	+/-	-	-	-	-	-	-
<i>Methylobacterium zatmanii</i>	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
<i>Methylobacterium aminovorans</i>	-	-	-	+	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-	-
<i>Methylobacterium radiotolerans</i>	+	+	+	-	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+
<i>Methylobacterium mesophilicum</i>	+	+	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+
<i>Methylobacterium fujisawaense</i>	+	+	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+
TK 0042, TK 0043	+	+	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+
TK 0038, TK 0037, TK 0044, TK 0045	+	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	+
TK 0040	+	+	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-	+

poration, and the complete tricarboxylic acid cycle is functional.

The type strain is TH-15, which was isolated from soil as a TMAH-utilizing bacterium by Urakami et al. in 1990 (35). This strain has all of the characteristics described above for the species. Its DNA base composition is 68.0 mol% G+C. The type strain has been deposited in the Japan Collection of Microorganisms, Institute of Physical and Chemical Research, as strain JCM 8240.

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