Transfer of *Pseudomonas aminovorans* (den Dooren de Jong 1926) to *Aminobacter* gen. nov. as *Aminobacter aminovorans* comb. nov. and Description of *Aminobacter aganoensis* sp. nov. and *Aminobacter niigataensis* sp. nov.

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We compared non-methane-, non-methanol-, and methylamine-utilizing bacteria, including *Pseudomonas aminovorans*, a tetramethylammonium-utilizing bacterium, and an *N,N*-dimethylformamide-utilizing bacterium. These bacteria are gram-negative, nonsporeforming, subpolarly flagellated, rod-shaped organisms. Reproduction occurs by budding. The DNA base compositions are 62 to 64 mol% guanine plus cytosine. The cellular fatty acids contain a large amount of C_{18:1} acid. The major hydroxy acid is 3-OH C_{12:0} acid. The major ubiquinone is ubiquinone Q-10. These bacteria are clearly separated from authentic *Pseudomonas* species (*Pseudomonas fluorescens* rRNA group) on the basis of utilization of methylamine, morphological and chemotaxonomic characteristics, DNA-DNA homology, and rRNA-DNA homology. They were divided into three subgroups on the basis of their physiological characteristics and DNA-DNA homology data. A new genus, *Aminobacter*, and three new species, *Aminobacter aminovorans* comb. nov., *Aminobacter aganoensis* sp. nov., and *Aminobacter niigataensis* sp. nov., are proposed. The type species of *Aminobacter* gen. nov. is *A. aminovorans*, with type strain JCM 7852. The type strain of *A. aganoensis* is strain TH-3 (= JCM 7854), and the type strain of *A. niigataensis* is strain DM-81 (= JCM 7853).

Although a large number of methane- and methylamine-utilizing bacteria and a large number of methanol- and methylamine-utilizing bacteria have been described (23, 29, 43-54), only a few non-methane-, non-methanol-, and methylamine-utilizing have been found to date (4, 10, 13, 22, 29, 32, 40-42). *Pseudomonas aminovorans* (4), *Pseudomonas* sp. strain MA (32), and *Pseudomonas* sp. strain MS (22) have been reported to be gram-negative methylamine-utilizing bacteria. These three bacteria were distinguished from methane- and methanol-utilizing bacteria on the basis of their failure to utilize methanol and methane. De Ley et al. (5) studied the rRNA cistron similarities of *Pseudomonas* and *Pseudomonas*-like strains and reported that the type strain of *P. aminovorans* was actually a member of the *Rhizobium loti* rRNA branch and not a member of the genus *Pseudomonas*. Furthermore, Green and Gillis (12) observed the budding multiplication of *P. aminovorans* strains and discussed the relationship of these organisms to *R. loti* and *Blastobacter* species. However, these authors did not make formal taxonomic proposals concerning *Pseudomonas aminovorans*. Recently, Tsuji et al. (39) described the results of a 16S rRNA sequence analysis performed to determine the phylogenetic relationships among methylotrophs; in this analysis they included methanol- and methylamine-utilizing *Methylophilus*, *Methylobacterium*, and *Hyphomicrobium* strains, but non-methane-, non-methanol-, and methylamine-utilizing bacteria were not studied.

Previously, we described the biodegradation of tetramethylammonium hydroxide (TMAH) and *N,N*-dimethylformamide (DMF) by bacteria (40, 42); in this work strains TH-3^T (T = type strain), DM-81^T, and TH-15, *Paracoccus amino-*

philus (41, 42), and *Paracoccus aminovorans* (41, 42) were described. Strains TH-3^T and DM-81^T were non-methane-, non-methanol-, monomethylamine-, dimethylamine-, and trimethylamine-utilizing, nonsporeforming, gram-negative, subpolarly flagellated, rod-shaped organisms with a ubiquinone Q-10 system, a cellular fatty acid composition which included a large amount of C_{18:1} acid, a hydroxy fatty acid composition which included a large amount of 3-hydroxy C_{12:0} acid, and a DNA base composition ranging from 62 to 64 mol% guanine plus cytosine (G+C) (40, 42). These characteristics were identical for *Pseudomonas aminovorans* JCM 7852^T, *Pseudomonas* sp. strain MA, and *Pseudomonas* sp. strain MS, as reported previously (47, 48).

In this study, we characterized five methylamine-utilizing bacteria, including strains TH-3^T and DM-81^T, and clarified the differentiation of these organisms from authentic *Pseudomonas* species; in this paper we propose the transfer of *Pseudomonas aminovorans* to a new genus, *Aminobacter*, as *Aminobacter aminovorans* and the establishment of two new species, *Aminobacter aganoensis* and *Aminobacter niigataensis*.

MATERIALS AND METHODS

Bacterial strains. The strains which we studied are listed in Table 1. Strain TH-3^T was isolated as a TMAH-utilizing bacterium, and strain DM-81^T was isolated as a DMF-utilizing bacterium (40, 42). *Pseudomonas aminovorans* JCM 7852^T (= NCIB 9039^T = NCTC 10684^T = ATCC 23314^T) (4), *Pseudomonas* sp. strain ATCC 23819 (= strain MA = NCIB 11590) (32), and *Pseudomonas* sp. strain ATCC 25262 (= strain MS = NCIB 11591) (22) are non-methane-, non-methanol-, and methylamine-utilizing bacteria (47, 48). *Pseudomonas diminuta* JCM 2788^T (= ATCC 11568^T =

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TABLE 1. Differentiating physiological characteristics of methylamine-utilizing bacteria

Strain	Nitrate reduction	Growth in peptone water	Utilization of:					Production of acid from L-arabinose oxidatively
			Dimethylamine	Formamide	N-Methylformamide	DMF	TMAH	
<i>Pseudomonas aminovorans</i> JCM 7852 ^T	—	+	—	—	—	—	—	+
<i>Pseudomonas</i> sp. strain ATCC 23819	—	+	—	—	—	—	—	+
<i>Pseudomonas</i> sp. strain ATCC 25262	—	+	—	—	—	—	—	+
TH-3 ^T	w ^a	w or —	+	—	—	—	+	—
DM-81 ^T	w	w or —	+	w	+	+	—	—

^a w, weak.

IAM 1513^T), *Pseudomonas vesicularis* IFO 12165^T (= ATCC 11426^T = IAM 12105^T), *Sphingomonas paucimobilis* (*Pseudomonas paucimobilis* [17, 59]) IFO 13935^T (= ATCC 29837^T = NCTC 11030^T), *Sphingomonas paucimobilis* (*Flavobacterium devorans* [17, 59]) NCIB 8195 (= ATCC 10829 = DSM 30198 = CCM 72), *Sphingomonas capsulata* (*Flavobacterium capsulatum* [17, 59]) NCIB 9890^T (= ATCC 14666^T = IFO 12533^T), *Pseudomonas aeruginosa* JCM 5962^T (= ATCC 10145^T = NCIB 8295^T = NCTC 10332^T = NRRL B-771^T), and *Pseudomonas fluorescens* NCIB 9046^T (= ATCC 13525^T = CCM 2115^T = DSM 50090^T = JCM 5963^T) were used as reference strains. Strains TH-3^T and DM-81^T and the three methylamine-utilizing bacteria resembled *Pseudomonas diminuta*, *Pseudomonas vesicularis*, *S. paucimobilis*, and *S. capsulata* on the basis of their cellular fatty acid compositions and quinone systems (16, 25, 26, 58, 59). *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* are typical species of the genus *Pseudomonas*. The bacterial strains were maintained on PYG agar (pH 7.0) (41).

Identification methods. PYG agar was used for preculturing and as the basal medium for strains TH-3^T and DM-81^T, *Pseudomonas aminovorans*, *Pseudomonas* sp. strain ATCC 23819, and *Pseudomonas* sp. strain ATCC 25262. Unless otherwise stated, the strains were cultivated aerobically in PYG broth at 30°C. Cell form, Gram reaction, motility, and multiplication were determined by using cells grown on PYG agar. Motility was observed by using the hanging drop method. Flagellar morphology and multiplication were determined by the staining method of Toda (37), using a light microscope, and by using an electron microscope when the organisms were negatively stained with 2% phosphotungstic acid (pH 7.0). Granules of poly-β-hydroxybutyrate in cells cultivated in medium containing 0.5% (wt/vol) DL-β-hydroxy-*n*-butyric acid (sodium salt) were stained by using the methods of Burdon (3). Biochemical and physiological characteristics were examined by previously described methods (41). Utilization of carbon compounds was determined by growth in liquid basal medium B (43) after 3 weeks of cultivation, and methanol was replaced with other carbon compounds as previously described (41). TMAH was added at a concentration of 0.15%.

Utilization of methanol, monomethylamine, dimethylamine, trimethylamine, trimethylamine-*N*-oxide, formamide, *N*-methylformamide, DMF, and TMAH by reference strains was determined as described above and previously (41).

Cellular fatty acid and hydroxy fatty acid compositions. Cells of reference strains cultivated aerobically for 1 day were used for the cellular fatty acid analysis. Cellular fatty

acid composition and hydroxy fatty acid composition were determined as described previously (49).

Quinone and quinone homologs. Cells of reference strains that were cultivated aerobically for 2 days were used for the quinone analysis. Quinone systems were determined as described previously (47). In the abbreviations used for ubiquinones, Q indicates ubiquinone, and the number indicates the number of isoprenoid units in the side chain (e.g., Q-9, Q-10, etc.).

DNA base composition. Cells that were cultivated aerobically for 2 days were used for the DNA analysis. DNA was extracted by using the method of Saito and Miura (31), and the G+C content was determined by reversed-phase high-performance liquid chromatography as described by Tamaoka and Komagata (36).

DNA-DNA hybridization. DNA-DNA hybridization was carried out at 68°C by using the method of Kaneko et al. (21). The DNAs of strains TH-3^T and DM-81^T, *Pseudomonas aminovorans* JCM 7852^T, *Pseudomonas diminuta* JCM 2788^T, and *S. paucimobilis* IFO 13935^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).

RESULTS

Phenotypic characteristics of methylamine-utilizing bacteria. The methylamine-utilizing bacteria (strains TH-3^T and DM-81^T, *Pseudomonas aminovorans* JCM 7852^T, *Pseudomonas* sp. strain ATCC 23819, and *Pseudomonas* sp. strain ATCC 25262) were gram-negative, nonsporeforming, rod-shaped, methylamine-utilizing organisms that were 0.5 to 0.9 by 1.0 to 3.0 μm and had rounded ends. Nonmucoid colonies were formed on PYG, nutrient, or methylamine-containing medium. The cells occurred singly or rarely in pairs, reproduced by budding, and were motile by means of subpolar flagella. The flagella of *Pseudomonas aminovorans* JCM 7852^T and strain DM-81^T are shown in Fig. 1. Granules of poly-β-hydroxybutyric acid were accumulated in the cells. Colonies were white to light yellow. Cells grew abundantly in nutrient broth and PYG broth. No water-soluble fluorescent pigment was produced on King B medium. The methyl red test and the Voges-Proskauer test in glucose phosphate broth (35) were negative. Indole and hydrogen sulfide were not produced. Hydrolysis of gelatin and starch was not observed. Ammonia was produced. Denitrification was negative. Litmus milk was not changed. All of the strains produced acids oxidatively from D-arabinose, D-xylose, D-glucose, D-mannose, D-fructose, galactose, maltose, su-

FIG. 1. Electron micrographs of negatively stained cells. Bar = 1 μ m. (A) *Pseudomonas aminovorans* JCM 7852^T. (B) Strain DM-81^T.

crose, trehalose, D-sorbitol, D-mannitol, inositol, and glycerol, but did not produce acids from lactose and soluble starch. Acids were not produced fermentatively. All strains grew at the expense of L-arabinose, D-xylose, D-glucose, D-mannose, D-fructose, D-galactose, maltose, sucrose, trehalose, D-sorbitol, D-mannitol, inositol, glycerol, succinic acid, acetic acid, monomethylamine, trimethylamine, and trimethylamine-*N*-oxide, but did not grow at the expense of lactose, soluble starch, citric acid, formic acid, ethanol, methanol, methane, or hydrogen. Growth factors were not

required as an essential supplement. All strains utilized ammonia, nitrate, urea, peptone, and methylamine as nitrogen sources. Oxidase and catalase were produced, but urease was not produced. Growth was observed at pH 6.0 to 8.0, but not at pH 5.0 and 9.0. All strains grew at 30 and 37°C, but not at 42°C. Growth did not occur in the presence of 3% NaCl (wt/vol). The biochemical and physiological characteristics that differed among the methylamine-utilizing bacteria are shown in Table 1.

Utilization of C₁ compounds by the reference strains. None

TABLE 2. Cellular fatty acid compositions of methylamine-utilizing bacteria and reference strains

Strain	Fatty acid composition (% of total acids)																	Unknown peak
	Straight-chain acids									Cyclopropane acids		3-Hydroxy acids			2-Hydroxy acids			
	C _{14:0}	C _{14:1}	C _{15:0}	C _{16:0}	C _{16:1}	C _{17:0}	C _{18:0}	C _{18:1}	C _{19:0}	C _{17:0}	C _{19:0}	3-OH C _{10:0}	3-OH C _{12:0}	3-OH C _{14:0}	2-OH C _{12:0}	2-OH C _{14:0}	2-OH C _{16:0}	
<i>Pseudomonas aminovorans</i> JCM 7852 ^{Ta}				9.8	0.2	0.2	7.6	54.6			3.0		0.3					22.5
<i>Pseudomonas</i> sp. strain ATCC 23819 ^a				8.4	1.6		3.4	64.5	0.2		3.2		0.5					18.2
<i>Pseudomonas</i> sp. strain ATCC 25262 ^a				7.4	4.2	0.4	7.7	53.5			4.5		0.1					22.2
TH-3 ^{Tb}				10.1	5.9		4.3	70.1					0.7	0.2				8.7
DM-81 ^{Tc}				7.3	6.7		3.1	72.8					0.9					9.2
<i>Pseudomonas diminuta</i> JCM 2788 ^T	0.8	1.6	2.0	18.9	1.8	2.1	0.4	63.9		4.5	1.0		3.0					
<i>Pseudomonas vesicularis</i> IFO 12165 ^T	2.8	0.2	0.2	20.1	5.7	0.1	1.1	64.8		0.4			4.6					
<i>S. paucimobilis</i> IFO 13935 ^T	1.6			12.0	2.8		0.8	68.4			0.1					14.2	0.1	
<i>S. paucimobilis</i> NCIB 8195	0.6			14.3	2.1		1.1	66.9			0.1					14.7	0.2	
<i>S. capsulata</i> NCIB 9890 ^T				10.3	3.9		0.5	73.0								12.2	0.1	
<i>Pseudomonas aeruginosa</i> JCM 5962 ^T	1.1			23.2	19.6		0.3	46.8		0.5	0.3	2.3	0.6		5.3			
<i>Pseudomonas fluorescens</i> NCIB 9046 ^T	1.6			30.8	35.2		1.5	22.0		0.1	0.2	3.0	1.1		4.5			

^a Data from reference 49.^b Data from reference 40.^c Data from reference 42.

of the reference strains utilized methanol, monomethylamine, dimethylamine, trimethylamine, trimethylamine-*N*-oxide, formamide, *N*-methylformamide, DMF, and TMAH.

Cellular fatty acid composition. The methylamine-utilizing bacteria (40, 42, 49), *Pseudomonas diminuta* JCM 2788^T, *Pseudomonas vesicularis* IFO 12165^T, *S. paucimobilis* IFO 13935^T and NCIB 8195, and *S. capsulata* NCIB 9890^T contained large amounts of straight-chain unsaturated C_{18:1} acid. The methylamine-utilizing bacteria did not contain straight-chain saturated C_{14:0} acid, but they did contain an identical unknown peak (Table 2). *Pseudomonas aeruginosa* JCM 5962^T and *Pseudomonas fluorescens* NCIB 9046^T contained large amounts of straight-chain saturated C_{16:0} acid and straight-chain unsaturated C_{16:1} and C_{18:1} acids. (Large amount was defined as 20% or more of the total acids.)

Hydroxy fatty acid composition. The methylamine-utilizing bacteria (40, 42, 49), *Pseudomonas diminuta*, and *Pseudomonas vesicularis* contained large amounts of 3-hydroxy C_{12:0} acid as a proportion of the total hydroxy fatty acids. *S. paucimobilis* and *S. capsulata* contained large amounts of 2-hydroxy C_{14:0} acid, and *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* contained large amounts of 3-hydroxy C_{10:0} and C_{12:0} acids, as well as 2-hydroxy C_{12:0} acid (Table 3).

Quinone system. The methylamine-utilizing bacteria (40, 42, 47), *Pseudomonas diminuta*, *Pseudomonas vesicularis*, *S. paucimobilis*, and *S. capsulata* had ubiquinone Q-10 systems (Table 3). In contrast, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* had ubiquinone Q-9 systems.

DNA base composition. The DNA base compositions of the methylamine-utilizing bacteria (40, 42) ranged from 62 to 64 mol% G+C (Table 4). The DNA base composition of *Pseudomonas aminovorans* 26^T of den Dooren de Jong (= JCM

7852^T) was reported to be 63.2 mol% by Mandel (24), but its value was determined to be 62.5 mol% in this study. The difference may have been due to the methods used for determination, as Mandel used the buoyant density method. The DNA base compositions of the reference strains ranged from 60 to 67 mol% G+C.

DNA-DNA hybridization. The levels of DNA-DNA homology among the methylamine-utilizing bacteria and reference strains are shown in Table 4. The strains tested were divided into four major groups (groups I through IV), and the similarity values between the groups were less than 20%. The five methylamine-utilizing bacteria (group I) were clearly distinguished from the reference strains. *Pseudomonas aminovorans*, *Pseudomonas* sp. strain ATCC 23819, and *Pseudomonas* sp. strain ATCC 25262 (group I-1) exhibited high levels (78 to 100%) of similarity to each other; strains TH-3^T (group I-2) and DM-81^T (group I-3) and the other methylamine-utilizing bacteria exhibited low levels (37 to 62%) of similarity to each other. On the other hand, *Pseudomonas diminuta* and *Pseudomonas vesicularis* (group II) were separated clearly from the other strains. *Pseudomonas diminuta* (group II-1) and *Pseudomonas vesicularis* (group II-2) exhibited low levels (34%) of similarity to each other. *S. paucimobilis* IFO 13935^T and NCIB 8195 (group III) exhibited high levels (84%) of similarity and were clearly separated from the other strains.

DISCUSSION

Pseudomonas sp. strain ATCC 23819 and *Pseudomonas* sp. strain ATCC 25262 were identified as *Pseudomonas aminovorans* by their phenotypic characteristics (Table 1) and by their high homology values (78 or 85%) in DNA-DNA

TABLE 3. Hydroxy fatty acid compositions and ubiquinone homologs of methylamine-utilizing bacteria and reference strains

Strain	3-Hydroxy acid			2-Hydroxy acid composition ^b			Ubiquinone homolog composition ^c				
	C _{10:0}	C _{12:0}	C _{14:0}	C _{12:0}	C _{14:0}	C _{16:0}	Q-7	Q-8	Q-9	Q-10	Q-11
<i>Pseudomonas aminovorans</i> JCM 7852 ^{Td}		100							2.5	97.0	0.5
<i>Pseudomonas</i> sp. strain ATCC 23819 ^d		100							5.8	93.7	0.5
<i>Pseudomonas</i> sp. strain ATCC 2526 ^d		100							6.6	93.2	0.2
TH-3 ^{Te}		78.8	21.2						0.3	99.7	
DM-81 ^{Tf}		100							1.6	98.4	
<i>Pseudomonas diminuta</i> JCM 2788 ^T	0.6	97.0	2.4					0.4	14.0	84.9	0.7
<i>Pseudomonas vesicularis</i> IFO 12165 ^T	0.5	95.2	4.3						7.5	91.7	0.8
<i>S. paucimobilis</i> IFO 13935 ^T					99.3	0.7		2.2	22.7	74.9	2.1
<i>S. paucimobilis</i> NCCIB 8195					98.7	1.3		1.2	14.2	84.4	0.2
<i>S. capsulata</i> NCIB 9890 ^T					99.2	0.8		1.0	5.6	92.1	1.3
<i>Pseudomonas aeruginosa</i> JCM 5962 ^T	69.9	30.1		100			0.3	6.2	92.8	0.7	
<i>Pseudomonas fluorescens</i> NCIB 9046 ^T	72.7	27.3		100				4.0	92.9	3.1	

^a Expressed as a percentage of the total 3-hydroxy acids.^b Expressed as a percentage of the total 2-hydroxy acids.^c Expressed as a percentage of the total ubiquinones.^d Data from references 47 and 49.^e Data from reference 40.^f Data from reference 42.

hybridization experiments (Table 4). Strains TH-3^T and DM-81^T should be included in the same genus to which *Pseudomonas aminovorans* belongs on the basis of their morphological characteristics and the following chemotaxonomic characteristics: cellular fatty acid composition (40, 42, 49), hydroxy fatty acid composition (40, 42, 49), quinone system (40, 42, 47, 60), DNA base composition (40, 42), and DNA-DNA homology. *Pseudomonas aminovorans*, which is a typical species of methylamine-utilizing bacteria, is included in Section V in *Bergey's Manual of Systematic Bacteriology* (27), which includes a number of *Pseudomonas* species whose natural relationships with well-characterized species of the genus are unknown. These methylamine-utilizing bacteria were distinguished clearly from the genus *Pseudomonas sensu stricto* (*Pseudomonas fluorescens* rRNA branch [8]) on the basis of the following characteristics: motility by subpolar flagella, multiplication by budding (12), utilization of methylamine, quinone system, cellular

fatty acid composition, hydroxy fatty acid composition, DNA-DNA homology, and rRNA-DNA homologies (5, 8). The following taxa have been reported to be gram-negative, nonpigmented, methylamine-utilizing bacteria: *Methylobacillus glycogenes* (46), *Methylophilus methylotrophus* (19), *Methylovorus glucosotrophus* (11), *Methylophaga* species (48), *Hyphomicrobium* species (14, 50), *Ancylobacter aquaticus* (45), *Paracoccus* species (41, 52), and *Thiobacillus versutus* (51). *Pseudomonas aminovorans* strains are distinguished from *Methylobacillus*, *Methylophilus*, *Methylovorus*, and *Methylophaga* species on the basis of multiplication mode, utilization of methanol and carbohydrates, quinone system (44, 47), cellular fatty acid composition (44, 49), hydroxy acid composition (46), and DNA base composition. Furthermore, *Pseudomonas aminovorans* strains metabolize C₁ compounds via the serine pathway (2, 54), whereas *Methylobacillus*, *Methylophilus*, *Methylovorus*, and *Methylophaga* strains metabolize these compounds by

TABLE 4. DNA base compositions and DNA-DNA homologies of methylamine-utilizing bacteria and reference strains

Strain	G+C content (mol%)	% of DNA-DNA homology with strain:					DNA-DNA homology group
		JCM 7852 ^T	TH-3 ^T	DM-81 ^T	JCM 2788 ^T	IFO 13935 ^T	
<i>Pseudomonas aminovorans</i> JCM 7852 ^T	62.5	100	37	37	6	5	I-1
<i>Pseudomonas</i> sp. strain ATCC 23819	63.2	78	45	44	— ^a	—	I-1
<i>Pseudomonas</i> sp. strain ATCC 25262	62.7	85	50	49	—	—	I-1
TH-3 ^T	63.8	58	100	60	—	—	I-2
DM-81 ^T	63.2	62	62	100	—	—	I-2
<i>Pseudomonas diminuta</i> JCM 2788 ^T	66.2	8	—	—	100	13	II-1
<i>Pseudomonas vesicularis</i> IFO 12165 ^T	65.9	6	—	—	34	11	II-2
<i>S. paucimobilis</i> IFO 13935 ^T	65.1	7	—	—	12	100	III
<i>S. paucimobilis</i> NCIB 8195	64.9	8	—	—	17	84	III
<i>S. capsulata</i> NCIB 9890 ^T	64.8	—	—	—	—	—	—
<i>Pseudomonas aeruginosa</i> JCM 5962 ^T	65.9	5	—	—	10	6	IV
<i>Pseudomonas fluorescens</i> NCIB 9046 ^T	60.2	—	—	—	—	—	—

^a —, not tested.

the ribulose monophosphate pathway (11, 19, 46, 48). *Pseudomonas aminovorans* strains are distinguished from *Hyphomicrobium* species on the basis of formation of hyphae, utilization of methanol and carbohydrates, quinone system, and hydroxy acid composition (14, 47, 50). *Pseudomonas aminovorans* strains are distinguished from *Ancylobacter* species on the basis of cell shape, utilization of methanol and carbon compounds, hydroxy acid composition (49), and DNA base composition (45). *Pseudomonas aminovorans* strains are distinguished from *Paracoccus* species on the basis of morphological characteristics, utilization of carbon compounds, hydroxy acid composition (49), and rRNA-DNA hybridization data (8, 18). *Pseudomonas aminovorans* strains are distinguished from *T. versutus* on the basis of morphological characteristics, utilization of carbon compounds, hydroxy acid composition, and DNA base composition (51). Furthermore, *Thiobacillus* species are characterized as β -purple bacteria on the basis of their 16S rRNA sequences (56). On the other hand, *Rhizobium* and *Agrobacterium* species, which are related to *Pseudomonas aminovorans* strains as determined by rRNA-DNA hybridization (5, 8), are characterized as α -purple bacteria (56). Therefore, allocation of these methylamine-utilizing bacteria to the genus *Methylobacillus*, *Methylophilus*, *Methylovorus*, *Methylophaga*, *Hyphomicrobium*, *Ancylobacter*, *Paracoccus*, or *Thiobacillus* is inappropriate. In 1982, Doronina et al. (9) isolated "*Blastobacter aminooxidans*," a budding methylamine-utilizing bacterium, from activated sludge. However, *Pseudomonas aminovorans* strains are distinguished from "*B. aminooxidans*" on the basis of motility, color of colonies, utilization of hydrogen and methanol, tolerance of NaCl, hydrolysis of gelatin and starch, metabolism of C_1 compounds, cellular fatty acid composition, and DNA base composition (9, 15, 38). Furthermore, the latter species is not on the Approved Lists of Bacterial Names (33). De Ley et al. (5) showed that *Pseudomonas aminovorans* NCTC 10684^T (= JCM 7852^T) is actually a member of the *R. loti* rRNA branch as determined by rRNA-DNA hybridization. However, *Pseudomonas aminovorans* strains are distinguished from *R. loti* on the basis of mucosity of colonies, utilization of methylamine, production of extracellular gum (20), hydroxy fatty acid composition (61), and thermal denaturation values versus 23S [³H]rRNA of *R. loti* NZP 2037 (5, 8). Also, the failure of representative *Pseudomonas aminovorans* strains to reduce acetylene has been described by Green and Gillis (12). Furthermore, these methylamine-utilizing bacteria were isolated from soils that were enriched with various amines, which served as sole carbon and energy sources. Therefore, allocation of these methylamine-utilizing bacteria to the genus *Rhizobium* is inappropriate. Green and Gillis (12) pointed out the resemblance based on phenotypic characteristics between *Pseudomonas aminovorans* strains and the genus *Blastobacter*, which contains heterotrophic rod-shaped budding bacteria that do not fix nitrogen. Although the genus *Blastobacter* contains *Blastobacter henricii* (type species), *Blastobacter aggregatus*, *Blastobacter capsulatus*, *Blastobacter denitrificans*, and *Blastobacter natatorius* as valid species (15, 34), no culture of the type species has been isolated (34). Furthermore, Trotsenko et al. stated in *Bergey's Manual* (38) that "future subdivision into two or more genera may become necessary." *Pseudomonas aminovorans* strains resemble *B. aggregatus* and *B. denitrificans* in motility and colony pigmentation, but are distinguished from *B. aggregatus* on the basis of production of acid from mannitol under aerobic conditions, utilization of ethanol and succinic acid, tolerance of NaCl, and DNA base

composition and from *B. denitrificans* on the basis of production of acid from mannitol under aerobic conditions, utilization of methanol, ethanol, and sucrose, tolerance of NaCl, and denitrification. Rothe et al. (30) reported that *B. aggregatus* DSM 111^T (= IFAM 10003^T) is more closely related to *Agrobacterium tumefaciens* DSM 30150 (S_{AB} , 0.84) than to *Rhizobium leguminosarum* ATCC 10004 (S_{AB} , 0.73) as determined by 16S rRNA cataloging performed to determine the phylogenetic positions of these organisms. *Agrobacterium tumefaciens* DSM 30150 is included in *Agrobacterium* cluster I as determined by rRNA-DNA hybridization (6). *Agrobacterium tumefaciens* is more closely related to *Rhizobium meliloti* and *R. leguminosarum* than to *R. loti* as determined by rRNA-DNA hybridization (5, 18). Therefore, *Pseudomonas aminovorans*, which is related to *R. loti* as determined by rRNA-DNA hybridization, should be distinguished from *B. aggregatus*. Green and Gillis (12) found that *Pseudomonas aminovorans* differed from *B. denitrificans* NCIB 12292^T on the basis of the rRNA-DNA hybridization data of Hirsch and Müller (15). Therefore, allocation of these methylamine-utilizing bacteria to the genus *Blastobacter* is inappropriate.

After considering the phenotypic, chemotaxonomic, and rRNA-DNA hybridization characteristics of the non-methane-, non-methanol-, and methylamine-utilizing, gram-negative, subpolarly flagellated, budding, rod-shaped organisms, we believe that it is appropriate to establish a new genus, *Aminobacter*, for these bacteria. The minimal characteristics for differentiating the genus *Aminobacter* from related genera are shown in Table 5. The DNA-DNA homology values for *Pseudomonas aminovorans* JCM 7852^T and strains TH-3^T and DM-81^T were approximately 37 to 62%, and these values may not be high enough to create new species. However, these strains were clearly distinguished on the basis of utilization of dimethylamine, *N*-methylformamide, DMF, and TMAH (Table 1). Therefore, we believe that strains TH-3^T and DM-81^T should be separated from *Pseudomonas aminovorans* and from each other at the species level.

We propose that *Pseudomonas aminovorans* should be transferred to the new genus *Aminobacter* as *Aminobacter aminovorans* and that the following two new species should be established: *Aminobacter aganoensis* for strain TH-3^T and *Aminobacter niigataensis* for strain DM-81^T. The minimal characteristics for differentiating *Aminobacter* species are shown in Table 1.

Pseudomonas diminuta and *Pseudomonas vesicularis* resemble each other and are distinguished from the genus *Pseudomonas sensu stricto* (*Pseudomonas fluorescens* rRNA branch [8]) on the basis of chemotaxonomic characteristics and DNA-DNA hybridization data. These two species are members of group IV as determined by the rRNA-DNA hybridization analysis of Palleroni et al. (28) and are included in Section IV of the genus *Pseudomonas* in *Bergey's Manual* (27). These findings were supported by the results of the rRNA-DNA homology analyses of De Vos and De Ley (7) and De Ley et al. (5) and by the results of a partial nucleic sequence analysis of 16S rRNA (56, 57, 59). We believe that *Pseudomonas diminuta* and *Pseudomonas vesicularis* represent a new genus and elsewhere will provide a description of this new genus.

Pseudomonas paucimobilis is included in Section V in *Bergey's Manual* (27). Several authors have suggested that *F. devorans* and *F. capsulatum* resemble *Pseudomonas paucimobilis* (1, 8, 58). Recently, Yabuuchi et al. proposed the new genus *Sphingomonas* and the new species *S. pauci-*

TABLE 5. Characteristics which differentiate the genus *Aminobacter* from related taxa

Genus or species	Color of colonies	Mucosity of colonies	Formation of hyphae	Denitrification	Tolerance of 3% NaCl	Acid produced from mannitol oxidatively	Utilization of:				G+C content (mol%)	Ubiquinone system	Major hydroxy acids
							Methanol	Ethanol	Sucrose	Succinic acid			
<i>Aminobacter</i>													
<i>Hyphomicrobium</i> ^a	Colorless	-	-	-	-	+	-	+	+	+	62-64	Q-10	3-OH C _{12:0}
<i>B. aggregatus</i> ^b	Colorless	-	+	+/-	-	-	+	+/-	-	-	59-65	Q-9	3-OH C _{14:0} , 3-OH C _{16:0}
<i>B. denitrificans</i> ^b	Colorless	-	-	-	+	-	-	+	+	-	60.4		
<i>Rhizobium</i> ^c	Colorless	+	-	-	+	-	+	+	-	+	64.8		
<i>Pseudomonas diminuta</i> ^d	Colorless	-	-	-	-	-	-	+/-	-	+/-	59-64	Q-10	3-OH C _{14:0} , 3-OH C _{12:0}
<i>Pseudomonas vesicularis</i> ^d	Colorless	-	-	-	-	-	-	+	-	+	66.2	Q-10	
<i>Sphingomonas</i> ^e	Yellow	-	-	-	-	-	-	-	+/-	-	65.9	Q-10	3-OH C _{12:0}
											64-66	Q-10	2-OH C _{14:0}

^a Data from references 14 and 50.^b Data from references 15 and 38.^c Data from references 20 and 61.^d Data from reference 27 and this study.^e Data from reference 59 and this study.

mobilis for *Pseudomonas paucimobilis* and *F. devorans* and the new species *S. capsulata* for *F. capsulatum* (17, 59). The chemotaxonomic characteristics found in this study support the proposal for the new genus *Sphingomonas*.

Description of *Aminobacter* Urakami, Araki, Oyanagi, Suzuki, and Komagata gen. nov. *Aminobacter* (Am.i.no.bac-ter. M.L.n. *aminum*, amine; M.L.n. *bacter*, rod, staff; M.L. masc.n. *Aminobacter*, amine rod). Nonsporeforming, gram-negative, rod-shaped, methylamine-utilizing organisms that are 0.5 to 0.9 by 1.0 to 3.0 μ m and have rounded ends. The cells occur singly (rarely in pairs) and are motile by means of subpolar flagella. Reproduction occurs by budding (12). Granules of poly- β -hydroxybutyric acid are accumulated in the cells. Colonies are white to light yellow. Cells grow abundantly in nutrient broth and PYG broth.

A water-soluble fluorescent pigment is not produced. The methyl red test and the Voges-Proskauer test are negative. Indole and hydrogen sulfide are not produced. Hydrolysis of gelatin and starch is not observed. Ammonia is produced. Denitrification is negative. Litmus milk is not changed. Acids are produced from sugars oxidatively, but not fermentatively. Monomethylamine, trimethylamine, trimethylamine-*N*-oxide, and sugars are utilized, but methanol, methane, and hydrogen are not utilized. Growth factors are not required as essential supplements. Ammonia, nitrate, urea, peptone, and methylamine are utilized as nitrogen sources. Oxidase and catalase are produced, but urease is not. Aerobic. Metabolism is strictly respiratory and not fermentative. Good growth occurs between pH 6.0 and 8.0, but growth does not occur above pH 9.0 and below pH 5.0. Good growth occurs at 30 and 37°C, but does not occur at 42°C. Growth does not occur in the presence of 3% sodium chloride.

The DNA base composition ranges from 62 to 64 mol% G+C. The cellular fatty acids include a large amount of straight-chain unsaturated C_{18:1} acid. The hydroxy acids include a large amount of 3-OH C_{12:0} acid. The ubiquinone system is ubiquinone Q-10.

The type species is *Aminobacter aminovorans*.

Description of *Aminobacter aminovorans* (den Dooren de Jong 1926) Urakami, Araki, Oyanagi, Suzuki, and Komagata comb. nov. *Aminobacter aminovorans* has all of the characteristics which define the genus. In addition, it has the characteristics described below. Growth occurs in peptone water. Nitrate is not reduced to nitrite. Acids are produced oxidatively from L-arabinose, D-xylose, D-glucose, D-mannose, D-fructose, D-galactose, maltose, sucrose, trehalose, D-sorbitol, D-mannitol, inositol, and glycerol, but are not produced from lactose and soluble starch. L-Arabinose, D-xylose, D-glucose, D-mannose, D-fructose, D-galactose, maltose, sucrose, trehalose, D-sorbitol, D-mannitol, inositol, glycerol, succinic acid, acetic acid, monomethylamine, trimethylamine, and trimethylamine-*N*-oxide are utilized, but lactose, soluble starch, citric acid, formic acid, ethanol, methanol, dimethylamine, formamide, *N*-methylformamide, DMF, TMAH, methane, and hydrogen are not utilized.

The type strain is strain JCM 7852 (= NCIB 9039 = ATCC 23314), which was isolated from soil enrichments containing various amines by de Dooren de Jong in 1926. This strain has all of the characteristics given above for the species. Its DNA base composition is 62.5 mol% G+C. The type strain has been deposited in the Japan Collection of Microorganisms, Institute of Physical and Chemical Research, as strain JCM 7852.

According to our results, *Pseudomonas* sp. strain ATCC 23819 (= strain MA = NCIB 11590) and *Pseudomonas* sp.

strain ATCC 25262 (= strain MS = NCIB 11591) should be identified as strains of *Aminobacter aminovorans*.

Description of *Aminobacter aganoensis* Urakami, Araki, Oyanagi, Suzuki, and Komagata sp. nov. *Aminobacter aganoensis* (a.ga.no. en'sis. M.L. neut. adj. *aganoensis*, coming from the Agano River, Niigata, Japan) has all of the characteristics which define the genus. In addition, it has the characteristics described below. Poor growth occurs in peptone water. Nitrate is reduced to nitrite weakly. Acids are produced oxidatively from D-xylose, D-glucose, D-mannose, D-fructose, D-galactose, maltose, sucrose, trehalose, D-sorbitol, D-mannitol, inositol, and glycerol, but are not produced from L-arabinose, lactose, and soluble starch. Formation of acids from D-glucose, D-mannose, maltose, sucrose, trehalose, D-sorbitol, D-mannitol, and inositol is weak. L-Arabinose, D-xylose, D-glucose, D-mannose, D-fructose, D-galactose, maltose, sucrose, trehalose, D-sorbitol, D-mannitol, inositol, glycerol, succinic acid, acetic acid, monomethylamine, dimethylamine, trimethylamine, trimethylamine-*N*-oxide, and TMAH are utilized, but lactose, soluble starch, citric acid, formic acid, ethanol, methanol, formamide, *N*-methylformamide, DMF, methane, and hydrogen are not utilized. Acetic acid is utilized weakly.

The type strain is strain TH-3. Strain TH-3^T has all of the characteristics given above for the species. Its DNA base composition is 63.8 mol% G+C. This strain was isolated as a TMAH-utilizing bacterium from soil by Urakami et al. (40). The type strain has been deposited in the Japan Collection of Microorganisms, Institute of Physical and Chemical Research, as strain JCM 7854.

Description of *Aminobacter niigataensis* Urakami, Araki, Oyanagi, Suzuki, and Komagata sp. nov. *Aminobacter niigataensis* (ni.i.ga.ta. en'sis. M.L. neut. adj. *niigataensis*, coming from the Niigata region of Japan) has all of the characteristics which define the genus. In addition, it has the characteristics described below. Poor growth occurs in peptone water. Nitrate is reduced to nitrite weakly. Acids are produced oxidatively from D-xylose, D-glucose, D-mannose, D-fructose, D-galactose, maltose, sucrose, trehalose, D-sorbitol, D-mannitol, inositol, and glycerol, but are not produced from L-arabinose, lactose, and soluble starch. L-Arabinose, D-xylose, D-glucose, D-mannose, D-fructose, D-galactose, maltose, sucrose, trehalose, D-sorbitol, D-mannitol, inositol, glycerol, succinic acid, acetic acid, monomethylamine, dimethylamine, trimethylamine, trimethylamine-*N*-oxide, *N*-methylformamide, and DMF are utilized, but lactose, soluble starch, citric acid, formic acid, ethanol, methanol, TMAH, methane, and hydrogen are not utilized. Formamide is utilized weakly.

The type strain is strain DM-81. Strain DM-81^T has all of the characteristics given above for the species. Its DNA base composition is 63.2 mol% G+C. This strain was isolated as a DMF-utilizing bacterium from soil by Urakami et al. (42). The type strain has been deposited in the Japan Collection of Microorganisms, Institute of Physical and Chemical Research, as strain JCM 7853.

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