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Acidomonas gen. nov., Incorporating *Acetobacter methanolicus* as *Acidomonas methanolica* comb. nov.

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A new genus of acidophilic, facultatively methylotrophic bacteria is described. These organisms are gram-negative, nonsporeforming, nonmotile, and rod shaped and grow at pH 2.0 to 5.5. These characteristics are unique among the methanol-utilizing bacteria. The deoxyribonucleic acid base composition is 63 to 65 mol% guanine plus cytosine. *Acetobacter methanolicus* TK 0705^T (T = type strain) is a typical strain in this group. These bacteria are distinguished from type and representative strains of *Acetobacter*, *Gluconobacter*, *Acidiphilium*, and *Thiobacillus* on the basis of deoxyribonucleic acid-deoxyribonucleic acid homology. A new genus, *Acidomonas*, is proposed to include this group of methylotrophic bacteria. The type species of the genus *Acidomonas* is *Acidomonas methanolica* comb. nov., with type strain TK 0705 (= IMET 10945).

We have reported previously (24, 25, 27) the grouping of gram-negative, methanol-utilizing bacteria into 11 groups on the basis of morphological characteristics, utilization of carbon compounds, cellular fatty acid composition, hydroxy fatty acid composition, ubiquinone systems, occurrence of squalene and steroids, and electrophoretic patterns of enzymes.

The group 7 bacteria of that previous study (24, 25) are acidophilic, facultatively methylotrophic, nonsporeforming, gram-negative, nonmotile, rod-shaped organisms. Steudel et al. (18) isolated a comparable bacterium, strain B58, which utilized methanol and grew at pH 4.0 to 4.2, and Uhlig et al. (21) named this organism *Acetobacter methanolicus* in 1986.

In this study we determined that the group 7 bacteria are identical to *Acetobacter methanolicus*, but we believe that the methylotrophic ability of these organisms demands a new genus, for which we propose the name *Acidomonas*.

MATERIALS AND METHODS

Bacterial strains. The strains which we studied are shown in Table 1. Below, names which do not appear on the Approved Lists of Bacterial Names (17) are enclosed in quotation marks. Cultures were maintained on peptone-yeast extract-malt extract agar containing 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, 1.0% glucose, and 2.0% agar; this medium was adjusted to pH 4.5 with 1 N HCl (PYM medium). *Acetobacter pasteurianus* TK 0724, TK 0728, and TK 0729 were received from J. De Ley as methanol-utilizing bacteria. *Thiobacillus novellus* TK 0901^T (T = type strain) and *Thiobacillus versutus* TK 3101^T were used as reference strains. *T. novellus* TK 0901^T was a member of the group 9 methanol-utilizing bacteria that we described previously, and *T. versutus* TK 3101^T was a member of the group 2 methylamine-utilizing bacteria which we described previously (24, 25). These two strains were maintained on peptone-yeast extract-glucose agar containing 0.5% peptone, 0.5% yeast extract, 0.5% glucose, and 2.0% agar; this medium was adjusted to pH 6.8 with 1 M NaOH.

Identification methods. PYM medium (pH 4.5) and medium C (24) containing methanol were used for preculture and basal media. Unless otherwise stated, all cultures were incubated at 30°C. Cell form, Gram reaction, and motility were investigated by using cells grown on PYM medium (pH 4.5) and medium C. Biochemical and physiological characteristics were investigated as reported previously (23). However, the pH of media was adjusted to 4.5. Ketogenic activity on polyalcohols (production of dihydroxyacetone from glycerol) was tested by using the method of Asai et al. (1). Acetic acid produced from ethanol was detected by gas chromatography. Nutritional requirements were determined by using a basal medium without yeast extract and the vitamin solution in medium C. Urease activity was observed on Christensen medium (2) for 1 week. Utilization of carbon compounds was determined in liquid basal medium C or medium B (22) (for *T. novellus* and *T. versutus*) after 3 weeks of cultivation, and methanol was replaced with other carbon compounds. Acetic acid, citric acid, lactic acid, monomethylamine, dimethylamine, and trimethylamine were added at concentrations of 0.15%. A total of 17 carbon compounds were added at concentrations of 0.5%.

Cellular fatty acid composition. Cellular fatty acid composition and hydroxy acid composition were determined as described previously (25).

Quinone system and quinone homologs. Quinone systems were determined as described previously (24).

DNA base composition. Deoxyribonucleic acid (DNA) was extracted by using the method of Saito and Miura (16), and guanine-plus-cytosine (G+C) content was determined by reverse-phase high-performance liquid chromatography as described by Tamaoka and Komagata (19).

DNA-DNA hybridization. DNA-DNA hybridization was carried out at 61°C by using the method of Kaneko et al. (13). DNA was labeled with [1′, 2′, 5-^{3}H]deoxycytidine triphosphate by the nick translation method (15), using Amersham Kit TRK 700 (Amersham International plc, Amersham, United Kingdom). DNA-DNA hybridization was carried out with five strains of the group 7 methanol-utilizing bacteria, seven strains of the genus *Acetobacter*, one strain of the genus *Gluconobacter*, four strains of the genus *Acidiphilium*, and two strains of the genus *Thiobacillus*.

RESULTS

Phenotypic characteristics of the group 7 bacteria. Five of the strains studied (strains TK 0701, TK 0702, TK 0703, and TK 0704 and *Acetobacter methanolicus* TK 0705^T) were

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TABLE 1. Bacterial strains studied

Strain	Source[a]	Reference(s)
Acetobacter methanolicus TK 0705^T	IMET 10945 (= Steudel et al. B58)	18, 21
"*Acetobacter sadu*" TK 0701	Urakami et al. BNS-25 (= JCM 3712 = FERM P-2664)	24, 25[b]
Isolate TK 0702	Urakami et al. I-2	24, 25
Isolate TK 0703	Urakami et al. T-101	24, 25
Isolate TK 0704	Urakami et al. EC-1	24, 25
Acidiphilium cryptum TK 0711^T	IFO 14242 (= Harrison et al. Lhe 2)	7, 9
Acidiphilium cryptum TK 0712	Harrison et al. m-5	7, 9
Acidiphilium cryptum TK 0713	Harrison et al. KG-4	7, 9
Acidiphilium angustum TK 0714^T	ATCC 35903	28
Acidiphilium facilis TK 0715^T	ATCC 35904	28
Acidiphilium sp. strain TK 0718	IFO 14243 (= Harrison et al. 44 het)	
Acetobacter aceti TK 0720^T	NCIB 8621	3
Acetobacter pasteurianus TK 0721^T	NCIB 12228	3
Acetobacter pasteurianus TK 0724	Gossele et al. LMG-1617 (= NICB 8620)	3, 5
Acetobacter pasteurianus TK 0728	Gossele et al. LMG-1701 (= VV3)	3, 5
Acetobacter pasteurianus TK 0729	Gossele et al. LMG-1804 (= NCPPB 462)	3, 5
Acetobacter xylinum TK 0731^T	NCIB 11664	12, 29
Acetobacter hansenii TK 0735^T	NCIB 8746	3, 5, 10
Acetobacter liquefaciens TK 0736^T	IAM 1834	3, 5, 10, 32
Acetobacter liquefaciens TK 0737	IAM 1835	3, 5, 10, 32
Acetobacter liquefaciens TK 0738	IAM 1836	3, 5, 10, 32
Gluconobacter oxydans TK 0740^T	NCIB 9013	3
Gluconobacter cerinus TK 0745^T	IFO 3267	11, 31
T. acidophilus TK 0719^T	ATCC 27807	6, 8
T. novellus TK 0901^T	NCIB 9113	20
T. versutus TK 3101^T	ATCC 25364	8

[a] Abbreviations for culture collections: ATCC, American Type Culture Collection, Rockville, Md.; FERM, Fermentation Research Institute, Agency of Industrial Science and Technology, Ibaragi, Japan; IAM, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; IMET, Zentralinstitut für Mikrobiologie und Experimentelle of Therapie, Akademie der Wissenschaften der DDR, Jena, East German Democratic Republic; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Wako-shi, Saitama, Japan; NCIB, National Collection of Industrial Bacteria, Aberdeen, United Kingdom; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom.
[b] Urakami, Japan patent 1,073,957, May 1981.

phenotypically similar, gram-negative, nonsporeforming, nonmotile, rod-shaped organisms that were 0.8 to 1.0 μm in diameter and 1.5 to 3.0 μm long. Colonies were white to light yellow on PYM medium (pH 4.5) and medium C. A water-soluble fluorescent pigment was not produced on King A and King B media adjusted pH to 4.5. Nitrate was not reduced to nitrite. The Voges-Proskauer test was negative. Indole and hydrogen sulfide (TSI medium adjusted pH 4.5) were not

TABLE 2. Utilization of carbon compounds by the group 7 methanol-utilizing bacteria and strains of *Acidiphilium cryptum*, *Thiobacillus* species, and *Acetobacter liquefaciens*

Strain	Utilization of:																								
	L-Arabinose	D-Xylose	D-Glucose	D-Mannose	D-Fructose	D-Galactose	Maltose	Sucrose	Lactose	Trehalose	D-Sorbitol	D-Mannitol	Inositol	Glycerol	Soluble starch	Pectin	Citric acid	Acetic acid	Lactic acid	Ethanol	Methanol	Monomethylamine	Dimethylamine	Trimethylamine	Methane	Hydrogen
Acetobacter methanolicus TK 0705^T	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	+	−	W[a]	−	+	+	−	−	−	−	−
"*Acetobacter sadu*" TK 0701	−	−	+	W	−	−	−	−	−	−	−	−	−	+	−	+	−	W	−	+	+	−	−	−	−	−
Isolate I-2 (= TK 0702)	−	−	+	W	−	−	−	−	−	−	−	−	−	+	−	+	−	W	−	W	+	−	−	−	−	−
Isolate T-101 (= TK 0703)	−	−	+	W	−	−	−	−	−	−	−	−	−	+	−	+	−	W	−	W	+	−	−	−	−	−
Isolate EC-1 (= TK 0704)	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	+	−	W	−	W	+	−	−	−	−	−
Acidiphilium cryptum TK 0711^T	+	+	+	W	+	+	−	+	−	−	+	+	+	+	−	W	−	−	−	−	−	−	−	−	−	−
Acidiphilium cryptum TK 0712	W	+	+	W	+	W	−	W	−	−	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−
Acidiphilium cryptum TK 0713	W	W	+	W	+	W	−	W	−	−	W	+	W	+	−	W	−	−	−	−	−	−	−	−	−	−
Acidiphilium sp. strain TK 0718	+	+	+	W	+	W	−	+	−	−	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−
T. acidophilus TK 0719^T	+	+	+	W	+	−	−	−	−	W	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−
T. novellus TK 0901^T	+	+	+	−	+	+	−	−	−	−	+	+	−	+	−	+	+	+	+	+	+	−	−	−	−	−
T. versutus TK 3101^T	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	W	+	+	+	−	+	−	−	−	−
Acetobacter liquefaciens TK 0736^T	−	−	−	−	+	+	−	W	−	−	−	+	−	+	−	W	−	W	+	W	−	−	−	−	−	−
Acetobacter liquefaciens TK 0737	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	W	−	−	−	−	−	−	−	−	−	−
Acetobacter liquefaciens TK 0738	−	−	−	−	+	+	−	W	−	−	−	+	−	+	−	W	−	W	+	W	−	−	−	−	−	−

[a] W, Weak.

produced. Hydrolysis of gelatin and starch was not observed. Ammonia was produced. Denitrification was not performed. Litmus milk was not changed. Dihydroxyacetone from glycerol was not produced. Acetic acid was produced from ethanol. Acid was produced from D-glucose oxidatively, but not from L-arabinose, D-xylose, D-mannose, D-fructose, D-galactose, maltose, sucrose, lactose, trehalose, D-sorbitol, D-mannitol, inositol, glycerol, or soluble starch. Acid was not produced fermentatively. All of the strains grew at the expense of methanol, ethanol, acetic acid, D-glucose, glycerol, and pectin, but did not grow at the expense of L-arabinose, D-xylose, D-fructose, D-galactose, maltose, sucrose, lactose, trehalose, D-sorbitol, D-mannitol, inositol, soluble starch, citric acid, lactic acid, monomethylamine, dimethylamine, trimethylamine, methane, or hydrogen. Some strains utilized D-mannose weakly (Table 2). Calcium pantothenate was required. Ammonia, nitrate, and urea were utilized by all of the strains as nitrogen sources. Urease, oxidase, and catalase were produced. Growth was observed between pH 2.0 and 5.5; the organisms did not grow at pH 1.5 and 6.0. The optimum cultural pH was 3.0 to 5.0, and the maximum specific growth rate was approximately 0.25 h^{-1}. All of the strains grew at 30 and 37°C, but did not grow at 42°C. Growth did not occur in the presence of 3% sodium chloride.

The group 7 bacteria were distinguished from the confusing *Acidiphilium*, *Thiobacillus*, and *Acetobacter* strains on the basis of utilization of carbon compounds (Table 2). All of the reference strains belonging to the genera *Acidiphilium*, Acetobacter, and *Gluconobacter* did not utilize methanol.

Cellular fatty acid composition. The reference strains belonging to the genera *Acidiphilium*, *Acetobacter*, and *Gluconobacter* contained large amounts of straight-chain unsaturated $C_{18:1}$ acid, as did the group 7 methanol-utilizing bacteria (25) and *Thiobacillus* strains (14, 25) (Table 3). The cellular fatty acid compositions of *Acetobacter* and *Gluconobacter* strains were determined by Yamada et al. (34). We confirmed these earlier results.

Hydroxy fatty acid composition. The group 7 methanol-utilizing bacteria had large amounts of 3-OH $C_{14:0}$ acid, 3-OH $C_{16:0}$ acid, 2-OH $C_{14:0}$ acid, and 2-OH $C_{16:0}$ acid (25). All of the *Acidiphilium* strains except *Acidiphilium facilis* TK 0715^T showed the presence of 3-hydroxy fatty acid, but not 2-hydroxy fatty acid, as shown in Table 3. All of the strains of the genera *Acetobacter* and *Gluconobacter* showed the presence of large amounts of 3-OH $C_{16:0}$ and 2-OH $C_{16:0}$ hydroxy acids and small amounts of 3-OH $C_{14:0}$ and 3-OH $C_{18:0}$ hydroxy acids, as well as 2-OH $C_{14:0}$ and 2-OH cyclopropane $C_{19:0}$ (2-OH $\Delta C_{19:0}$) acids.

Quinone system. The group 7 methanol-utilizing bacteria had the Q-10 ubiquinone system along with ubiquinone Q-9 and minor ubiquinone Q-11 components (24). The reference strains belonging to the genera *Acidiphilium*, *Thiobacillus* (14, 24), and *Gluconobacter* had the Q-10 ubiquinone system, along with minor ubiquinone Q-9 and Q-11 components. *Acetobacter* strains were divided into two types (Q-9 and Q-10 types), as shown in Table 3. The same results for quinone systems in *Acetobacter* species (except *Acetobacter hansenii*) and *Gluconobacter* species were reported by Yamada et al. (30).

DNA base composition. The DNA base compositions of five strains of the group 7 methanol-utilizing bacteria ranged from 63.7 to 64.6 mol% G+C. The DNA compositions of the reference strains are shown in Table 4. The DNA base compositions of *Acetobacter methanolicus*, *Acidiphilium cryptum*, *Acidiphilium angustum*, and *Acetobacter aceti*

TABLE 3. Cellular fatty acid compositions and ubiquinone homologs of strains in the genera *Acidiphilium*, *Acetobacter*, and *Gluconobacter* and *T. acidophilus*

Strain	Fatty acid composition (%)																Ubiquinone homologs				
	Straight-chain acids							3-Hydroxy acids			2-Hydroxy acids										
	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{19:0}$	$C_{19:0}$ cyclopropane acid	3-OH $C_{14:0}$	3-OH $C_{16:0}$	3-OH $C_{18:0}$	2-OH $C_{12:0}$	2-OH $C_{14:0}$	2-OH $C_{16:0}$	2-OHΔ $C_{19:0}$	Q-7	Q-8	Q-9	Q-10	Q-11
Acidiphilium cryptum TK 0711^T	1.0		1.7	0.6	0.5	7.0	83.0	2.0	1.3	2.9									2.1[a]	96.2	1.7
Acidiphilium cryptum TK 0712	0.3		2.0	1.0	0.3	6.8	83.1	1.8	1.0	2.7									2.6	94.6	2.8
Acidiphilium cryptum TK 0713			2.6	2.3	0.8	8.0	79.9	2.4	2.0	2.0									3.0	94.9	2.1
Acidiphilium angustum TK 0714^T	0.5		6.4	3.3		2.0	73.8	1.2	4.7	3.0	0.2	4.9					2.2	10.4	87.2	0.2	
Acidiphilium facilis TK 0715^T	0.1		9.7	0.1		0.6	70.2	0.2	6.4	2.0	1.1	2.3			7.3		3.0	12.6	83.9	0.5	
Acidiphilium sp. strain TK 0718			2.6	0.6		7.4	85.3	0.9	1.8	1.4								3.2	95.3	1.5	
Acetobacter aceti TK 0720^T	2.4		17.0			5.1	60.5		0.2	0.7	1.7	0.8		5.5	6.1		0.7	18.8	78.1	2.4	
Acetobacter pasteurianus TK 0722^T	2.8		11.0	0.1		2.4	60.9		0.5	1.0	4.1	1.2		7.3	7.2	1.5	0.5	16.3	81.0	2.2	
Acetobacter pasteurianus TK 0724	5.3	0.2	7.7	0.1		3.0	65.4		0.7	0.7	2.7	0.6		0.8	11.1	1.7		15.8	78.4	5.8	
Acetobacter pasteurianus TK 0728	0.3		6.8			2.0	65.7			0.6	1.8	0.5		7.7	14.6			11.6	82.7	5.7	
Acetobacter pasteurianus TK 0729	5.9	0.2	9.1			3.1	62.7		0.5	0.7	2.7	0.8		1.3	11.0	2.0		15.4	81.0	3.6	
Acetobacter xylinum TK 0731^T	2.2		10.6			1.4	69.8			0.9	2.9		0.4	4.0	7.8			0.5	7.8	91.3	0.4
Acetobacter hansenii TK 0735^T	2.9	0.5	14.9	0.3	1.9	1.4	60.3		0.1	2.0	5.5	0.4		3.2	6.6			1.9	15.9	82.1	0.1	
Acetobacter liquefaciens TK 0736^T	5.5		9.6		0.7	2.9	65.6		1.0	1.3	2.7	0.5		2.3	6.6	1.3			7.1	92.5	0.4	
Acetobacter liquefaciens TK 0737	0.1		14.1			1.1	59.5	0.1		1.2	3.5	1.2			13.5	5.7			3.0	96.6	0.4	
Acetobacter liquefaciens TK 0738	6.7		10.7		0.3	3.7	66.8	0.1	0.6	1.7	2.6	0.5		0.3	6.0				9.8	89.9	0.3	
Gluconobacter oxydans TK 0740^T			9.2			2.1	68.7		0.2	1.6	4.4	0.6			11.0	2.2			2.4	97.3	0.3	
Gluconobacter cerinus TK 0745^T	0.1	0.1	11.4			7.4	62.5		0.1	1.2	2.0	2.6			5.9	6.7			5.5	92.1	2.4	
T. acidophilus TK 0719^T																			1.8	96.7	1.5	

[a] The values are percentages of the total ubiquinones.

TABLE 4. DNA base compositions, cellular fatty acid compositions, hydroxy fatty acid compositions,[k] and ubiquinone types of the group 7 methanol-utilizing bacteria and strains in the genera *Acidiphilium*, *Acetobacter*, *Gluconobacter*, and *Thiobacillus*

Strain	G+C content (mol%)		Major cellular fatty acid	Major 3-hydroxy fatty acid(s)	Major 2-hydroxy fatty acid	Quinone type
	This study	Other study				
Acetobacter methanolicus TK 0715^T	64.4	62.3[a]	$C_{18:1}$[b]	$C_{16:0}$ ($C_{14:0}$)[b]	$C_{16:0}$ ($C_{14:0}$)[b]	Q-10[c]
"*Acetobacter sadu*" TK 0701	64.2	64.6[d]	$C_{18:1}$[b]	$C_{16:0}$ ($C_{14:0}$)[b]	$C_{16:0}$ ($C_{14:0}$)[b]	Q-10[c]
Isolate I-2 (= TK 0702)	64.1	65.1[d]	$C_{18:1}$[b]	$C_{16:0}$ ($C_{14:0}$,$C_{10:0}$)[b]	$C_{16:0}$ ($C_{14:0}$)[b]	Q-10[c]
Isolate T-101 (= TK 0703)	63.7	63.7[d]	$C_{18:1}$[b]	$C_{16:0}$ ($C_{14:0}$)[b]	$C_{16:0}$ ($C_{14:0}$)[b]	Q-10[c]
Isolate EC-1 (= TK 0704)	64.6	64.1[d]	$C_{18:1}$[b]	$C_{16:0}$ ($C_{14:0}$)[b]	$C_{16:0}$ ($C_{14:0}$)[b]	Q-10[c]
Acidiphilium cryptum TK 0711^T	66.2	69.4[e]	$C_{18:1}$	$C_{14:0}$		Q-10
Acidiphilium cryptum TK 0712		70.0[e]	$C_{18:1}$	$C_{14:0}$		Q-10
Acidiphilium cryptum TK 0713		68.1[e]	$C_{18:1}$	$C_{14:0}$		Q-10
Acidiphilium angustum TK 0714^T	62.3	67[f]	$C_{18:1}$	$C_{18:0}$ ($C_{14:0}$)		Q-10
Acidiphilium facilis TK 0715^T	64.0	65[f]	$C_{18:1}$	$C_{14:0}$, $C_{18:0}$ ($C_{16:0}$)	$C_{16:0}$	Q-10
Acidiphilium sp. strain TK 0718	66.2		$C_{18:1}$	$C_{14:0}$		Q-10
Acetobacter aceti TK 0720^T	56.5	58.6[g]	$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$,$C_{18:0}$)	$C_{16:0}$ ($C_{14:0}$)	Q-9
Acetobacter pasteurianus TK 0722^T	53.0		$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$,$C_{18:0}$)	$C_{16:0}$ ($C_{14:0}$, $\Delta C_{19:0}$)	Q-9
Acetobacter pasteurianus TK 0724	57.2	58.0[h]	$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$,$C_{18:0}$)	$C_{16:0}$ ($\Delta C_{19:0}$)	Q-9
Acetobacter pasteurianus TK 0728			$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$)	$C_{16:0}$ ($C_{14:0}$)	Q-9
Acetobacter pasteurianus TK 0729	56.9		$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$,$C_{18:0}$)	$C_{16:0}$ ($\Delta C_{19:0}$)	Q-9
Acetobacter xylinum TK 0731^T	61.5		$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$,$C_{18:0}$)	$C_{16:0}$ ($C_{14:0}$)	Q-10
Acetobacter hansenii TK 0735^T	58.5	59.7[h]	$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$)	$C_{16:0}$ ($C_{14:0}$)	Q-10
Acetobacter liquefaciens TK 0736^T	64.2	64.5[g]	$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$,$C_{18:0}$)	$C_{16:0}$ ($C_{14:0}$, $\Delta C_{19:0}$)	Q-10
Acetobacter liquefaciens TK 0737		66.2[g]	$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$,$C_{18:0}$)	$C_{16:0}$ ($\Delta C_{19:0}$)	Q-10
Acetobacter liquefaciens TK 0738		64.0[g]	$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$,$C_{18:0}$)	$C_{16:0}$	Q-10
Gluconobacter oxydans TK 0740^T	60.1	60.6[g]	$C_{18:1}$	$C_{16:0}$ ($C_{14:0}$,$C_{18:0}$)	$C_{16:0}$ ($\Delta C_{19:0}$)	Q-10
Gluconobacter cerinus TK 0745^T	55.8	55.7[g]	$C_{18:1}$	$C_{14:0}$, $C_{16:0}$, $C_{18:0}$	$C_{16:0}$, $\Delta C_{19:0}$	Q-10
T. acidophilus TK 0719^T		62.9-63.2[i]	$C_{18:1}$[i]	$C_{14:0}$[i]		Q-10
T. novellus TK 0901^T	66.9	67.7[d]	$C_{18:1}$[b]	$C_{14:0}$ ($C_{10:0}$)		Q-10[c]
T. versutus TK 3101^T	67.2	68.0[j]	$C_{18:1}$[b]	$C_{10:0}$ ($C_{14:0}$,$C_{16:0}$)		Q-10[c]

[a] Data from reference 21.
[b] Data from reference 25.
[c] Data from reference 24.
[d] Data from reference 26.
[e] Data from reference 7.
[f] Data from reference 28.
[g] Data from reference 33.
[h] Data from reference 4.
[i] Data from reference 14.
[j] Data from reference 8.
[k] Parentheses indicate minor components.

determined in this study were different from the results reported by other workers (6, 20, 28, 33). The differences among the results may be ascribed to assay methods.

DNA-DNA homologies. The levels of DNA-DNA homology among the group 7 methanol-utilizing bacteria and the reference strains clearly indicate a separation of the group 7 strains from the strains belonging to the genera *Acidiphilium*, *Acetobacter*, and *Gluconobacter*, as shown in Table 5. Furthermore, five strains (TK 0701, TK 0702, TK 0703, TK 0704, TK 0705^T) showed high levels of similarity to each other. The reference strains belonging to the genera *Acidiphilium*, *Acetobacter*, and *Gluconobacter* showed low levels of similarity each other. An exception was *Acidiphilium* sp. strain TK 0718 and *Acidiphilium crytpum*, which showed a relatively high similarity value.

DISCUSSION

The five strains of group 7 methanol-utilizing bacteria grew at pH 2.0 to 5.5, and this characteristic is unique among the methanol-utilizing bacteria. They shared almost the same phenotypic characteristics, as well as the same cellular fatty acid composition (25), hydroxy fatty acid composition (25), ubiquinone system (24), and DNA base composition. The strains which we studied showed high levels of similarity in

TABLE 5. DNA-DNA homologies among the group 7 methanol-utilizing bacteria and strains in the genera *Acetobacter*, *Gluconobacter*, *Acidiphilium*, and *Thiobacillus*

Strain	% DNA-DNA homology with strain:			
	TK 0705^T	TK 0720	TK 0740	TK 0711
Acetobacter methanolicus TK 0705^T	100	13	8	10
"*Acetobacter sadu*" TK 0701	107	14	10	14
Isolate I-2 (= TK 0702)	95	13	12	16
Isolate T-101 (= TK 0703)	89	12	9	11
Isolate EC-1 (= TK 0704)	101	14	11	14
Acetobacter aceti TK 0720^T	13	100	8	3
Acetobacter pasteurianus TK 0722^T	7	11	13	5
Acetobacter pasteurianus TK 0724	21	18	15	10
Acetobacter pasteurianus TK 0729	10	12	10	16
Acetobacter xylinum TK 0731^T	18	18	13	10
Acetobacter hansenii TK 0735^T	19	14	14	16
Acetobacter liquefaciens TK 0736^T	24	12	14	22
Gluconobacter oxydans TK 0740^T	15	15	100	8
Acidiphilium cryptum TK 0711^T	11	2	2	100
Acidiphilium sp. strain TK 0718	12	2	3	58
Acidiphilium angustum TK 0714^T	14	6	5	27
Acidiphilium facilis TK 0715^T	11	3	4	15
T. novellus TK 0901^T	14	4	4	25
T. versutus TK 3101^T	16	3	6	19

TABLE 6. Characteristics which differentiate the genus *Acidomonas* from related genera

Genus	Type species	Flagellation	Utilization of:			G+C content (mol%)	Major hydroxy acid(s)	Quinone system
			Methanol	D-Mannitol	Lactic acid			
Acidomonas	*Acidomonas methanolica*	−	+	−	−	63–66	3-OH $C_{16:0}$, 2-OH $C_{16:0}$	Q-10
Acetobacter	*Acetobacter aceti*	+ (Peritrichous)	−	+	+	56.5[a]	3-OH $C_{16:0}$, 2-OH $C_{16:0}$	Q-9
Gluconobacter	*Gluconobacter oxydans*	+ (Polar)	−	+	−	60.1[a]	3-OH $C_{16:0}$, 2-OH $C_{16:0}$	Q-10
Acidiphilium	*Acidiphilium cryptum*	+ (Polar)	−	+	−	66.2[a]	3-OH $C_{14:0}$	Q-10

[a] Data for the type strain in this study.

DNA-DNA hybridization tests with the type strain of *Acetobacter methanolicus* and "*Acetobacter sadu*" and a clear separation from the reference strains. Therefore, these bacteria fall into a single taxon and can be identified phenotypically and genotypically as *Acetobacter methanolicus*.

The group 7 bacteria are distinguished from *Acidiphilium* strains, *Thiobacillus acidophilus*, *T. novellus*, *T. versutus*, and *Gluconobacter* and *Acetobacter* strains by phenotypic and chemotaxonomic characteristics (Tables 2, 4, and 5) (1, 7, 28, 30, 32). Gossele et al. (5) reported that some strains of *Acetobacter pasteurianus* utilize methanol, but the three strains which these authors reported to utilize methanol did not utilize methanol in this study.

After considering the phenotypic and chemotaxonomic characteristics of these acidophilic, facultatively methylotrophic, nonmotile, gram-negative, rod-shaped organisms, we believe that it is appropriate to separate *Acetobacter methanolicus* from *Acetobacter* and establish a new genus, *Acidomonas*, for this group of bacteria. We designate *Acidomonas methanolica* comb. nov. as the type species of this genus. The type strain is *Acidomonas methanolica* TK 0705. The minimal characteristics for differentiating the genus *Acidomonas* from related genera are shown in Table 6.

Description of *Acidomonas* gen. nov. *Acidomonas* (A. ci. do. mo'nas. Gr. adj. *acid*, acid; Gr. n. *monas*, unit, monad; M. L. fem. n. *Acidomonas*, acidophilic monad). The type species is *Acidomonas methanolica* (Uhlig et al. 1986) Urakami, Tamaoka, Suzuki, and Komagata comb. nov. The essential characteristics of the genus are given in Table 6 and in the description of the single species, *Acidomonas methanolica*.

Description of *Acidomonas methanolica* (Uhlig, Karbaum, and Steudel 1986) Urakami, Tamaoka, Suzuki, and Komagata comb. nov. *Acidomonas methanolica* (Synonym, *Acetobacter methanolicus* Uhlig, Karbaum, and Steudel 1986). The characteristics of the species are those reported by Uhlig et al. (21) and those determined in this study.

The cells are nonsporeforming, gram-negative rods with rounded ends that are 0.8 to 1.0 by 1.5 to 3.0 μm. The cells occur singly, rarely in pairs, and are nonmotile.

Colonies on PYM medium (pH 4.5) are shiny, smooth, raised, entire, white to light yellow, and 2 to 3 mm in diameter after 3 days at 30°C. A water-soluble fluorescent pigment is not produced. Nitrate is not reduced to nitrite. The Voges-Proskauer test is negative. Indole and hydrogen sulfide are not produced. Hydrolysis of gelatin and starch is not observed. Ammonia is produced. Litmus milk is not changed. Dihydroxyacetone is not produced from glycerol. Acetic acid is produced from ethanol. Acid is produced from D-glucose oxidatively, but not from L-arabinose, D-xylose, D-mannose, D-fructose, D-galactose, maltose, sucrose, lactose, trehalose, D-sorbitol, D-mannitol, inositol, glycerol, or soluble starch. Acid is not produced fermentatively. Utilizes methanol, ethanol, acetic acid, D-glucose, glycerol, and pectin as sole carbon sources for energy and growth, but does not utilize L-arabinose, D-xylose, D-fructose, D-galactose, maltose, sucrose, lactose, trehalose, D-sorbitol, D-mannitol, inositol, soluble starch, citric acid, lactic acid, methylamine, methane, or hydrogen. Some strains utilize D-mannose weakly (Table 2). Calcium panthothenate is required. Ammonia, nitrate, and urea are utilized as nitrogen sources. Urease, oxidase, and catalase are produced. Aerobic. Metabolism is strictly respiratory and not fermentative.

Growth occurs between pH 2.0 and 5.5. Good growth occurs between pH 3.0 and 5.0. Growth does not occur above pH 6.0 and below pH 1.5. Good growth occurs at 30°C 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 is 64.4 mol% G+C (type strain) (variable from 63 to 65 mol% G+C [Table 4]). The cellular fatty acids are composed of a large amount of straight-chain unsaturated $C_{18:1}$ acid and small amounts of straight-chain saturated $C_{15:0}$ acid, $C_{16:0}$ acid, $C_{17:0}$ acid, $C_{18:0}$ acid, and $C_{19:0}$ acid, straight-chain unsaturated $C_{16:1}$ acid, and $C_{19:0}$ cyclopropane acid. The hydroxy acids are composed of large amounts of 3-OH $C_{14:0}$ acid, 3-OH $C_{16:0}$ acid, 2-OH $C_{14:0}$ acid, and 2-OH $C_{16:0}$ acid. The ubiquinone system is Q-10, along with ubiquinone Q-9 and minor ubiquinone Q-11 components.

The type strain is strain TK 0705 (= IMET 10945), which was isolated by Steudel et al. in 1980. This strain has been deposited in the Zentralinstitut für Mikrobiologie und Experimentell Therapie as strain IMET 10945.

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