Paracoccus alcaliphilus sp. nov., an Alkaliphilic and Facultatively Methylophilic Bacterium

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Alkaliphilic facultatively methanol-utilizing bacteria were investigated with respect to their phenotypic and chemotaxonomic characteristics. These bacteria were gram-negative, nonsporeforming, nonmotile, coccoid or short rod-shaped organisms, grew at pH 7.0 to 9.5, and did not grow below pH 6.5 and above pH 10.0. The deoxyribonucleic acid (DNA) base composition was 64 to 66 mol% guanine plus cytosine. These bacteria resemble *Paracoccus denitrificans* with respect to morphological and chemotaxonomic characteristics, but are distinguished from *P. denitrificans* by physiological characteristics and DNA-DNA homology. These methanol-utilizing bacteria are described as a new species, *Paracoccus alcaliphilus* Urakami, Tamaoka, Suzuki, and Komagata. The type strain of *P. alcaliphilus* sp. nov. is strain TK 1015 (= JCM 7364).

Previously, we (15, 16, 18, 19) reported the grouping of gram-negative, methanol-utilizing bacteria on the basis of morphological characteristics, utilization of carbon compounds, deoxyribonucleic acid (DNA) base composition, cellular fatty acid composition, hydroxy fatty acid composition, ubiquinone system, occurrence of squalene and steroids, and electrophoretic properties of enzymes and divided these bacteria into 11 groups.

The group 10 methanol-utilizing bacteria and the group 8 methanol-utilizing bacteria that we described (15, 16, 18, 19) are facultatively methylophilic, nonsporeforming, gram-negative, nonmotile, coccoid or short rod-shaped organisms with a Q-10 ubiquinone system, a cellular fatty acid composition consisting of a large amount of C_{18:1} acid (type B), and a hydroxy fatty acid composition consisting of a large amount of 3-OH C_{10:0} acid and 3-OH C_{14:0} acid. However, these two groups are distinguished from each other by the pH range for growth and DNA-DNA homologies. The group 8 bacteria have previously been identified as *Paracoccus denitrificans* (8, 15, 16, 18, 19). *P. denitrificans* strains do not grow under alkaline conditions, but the group 10 methanol-utilizing bacteria grow even at pH 9.0.

In this study we characterized the group 10 methanol-utilizing bacteria and *Paracoccus* strains and established a new species, *Paracoccus alcaliphilus*, for the group 10 methanol-utilizing bacteria.

MATERIALS AND METHODS

Bacterial strains. The strains which we studied are shown in Table 1. The group 10 bacteria were maintained on medium D (pH 9.0) (15). *P. denitrificans* strains were maintained on medium B (15) or PYG agar medium containing 0.5% peptone, 0.5% yeast extract, 0.5% glucose, and 0.2% agar; this medium was adjusted to pH 7.0 with 1 N NaOH.

Identification methods. Medium D agar (pH 9.0) (15) containing methanol was used for the group 10 bacteria. Medium B agar (pH 7.1) (15) containing methanol and PYG agar medium were used for preculture and basal media for *P. denitrificans* strains. Unless otherwise stated, the strains were cultivated at 30°C. Cell form, Gram reaction, and motility were determined by using cells grown on each basal

medium. 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 the method of Burdon (3). Biochemical and physiological characteristics were examined for comparison with other aerobic bacteria by using previously described methods (14, 17). Media adjusted to pH 9.0 with a 10% Na₂CO₃ solution were used for the group 10 bacteria. Utilization of carbon compounds was determined in liquid basal medium B or medium D, and methanol or D-glucose was replaced with other carbon compounds as previously described (17).

DNA base composition. Cells cultivated in each of the basal media at 30°C for 2 to 3 days with shaking were used for the DNA analysis. DNA was extracted by using the method of Saito and Miura (10), and the guanine-plus-cytosine (G+C) content was determined by reverse-phase high-performance liquid chromatography as described by Tamaoka and Komagata (13).

DNA-DNA hybridization. DNA-DNA hybridization was carried out at 61°C by using the method of Kaneko et al. (7). DNAs from isolate TK 1015^T (T = type strain) of the group 10 methanol-utilizing bacteria and *P. denitrificans* ATCC 17441^T were labeled with [1',2',5'-³H]-deoxycytidine triphosphate by using the nick translation method and kit TRK 700 (Amersham International plc, Amersham, United Kingdom). DNA-DNA hybridizations were carried out with three strains of group 10 methanol-utilizing bacteria and three strains of *P. denitrificans*.

RESULTS

Phenotypic characteristics of the group 10 bacteria. Phenotypic characteristics of the group 10 bacteria are shown in Tables 2 and 3. All of the strains were gram negative, nonsporeforming, nonmotile, coccoid or short rod-shaped organisms, measuring 0.6 to 1.0 μm in diameter and 0.8 to 2.0 μm long. Granules of poly-β-hydroxybutyric acid accumulated in the cells. Colonies were white or light yellow on medium D (pH 9.0). Poor growth in nutrient broth and peptone water was observed. A water-soluble fluorescent pigment was not produced on King A and King B media adjusted to pH 9.0. Nitrate was reduced to nitrite. The Voges-Proskauer test in glucose phosphate broth (12) adjusted to pH 9.0 was negative. Production of indole in 1% tryptone broth adjusted to pH 9.0 and production of hydro-

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TABLE 1. Bacterial strains studied

Strain	Other strain designations ^a	Reference(s)
Group 10 methanol-utilizing bacteria		
Isolate TK 1001	Urakami 0-3	
Isolate TK 1002	Urakami 0-9	
Isolate TK 1003	Urakami 0-13	
Isolate TK 1004	Urakami 0-17	
Isolate TK 1005	Urakami 0-19	
Isolate TK 1006	Urakami 0-20	
Isolate TK 1007	Urakami 0-74	
Isolate TK 1008	Urakami 0-75	
Isolate TK 1009	Urakami 0-78	
Isolate TK 1010	Urakami 0-83	
Isolate TK 1011	Urakami 0-88	
Isolate TK 1012	Urakami 0-89	
Isolate TK 1013	Urakami 0-90	
Isolate TK 1014	Urakami 0-91	
Isolate TK 1015 ^T	Urakami 0-100	
Group 8 methanol-utilizing bacteria		
<i>P. denitrificans</i> ATCC 17441 ^T	DSM 65 ^T , IAM 12479 ^T , NCIB 11627 ^T	4, 11
<i>P. denitrificans</i> ATCC 13543	CCM 982, NRRL 3784	4, 20
<i>P. denitrificans</i> IFO 13301	ATCC 19367, DSM 413, NCIB 8944, NRRL 3785	4, 20
<i>P. denitrificans</i> NCIB 9722	CCM 1396, DSM 415	4, 20
<i>P. denitrificans</i> DSM 1403		9
<i>P. denitrificans</i> DSM 1404		9
<i>P. denitrificans</i> DSM 1405		9
<i>P. denitrificans</i> DSM 1406		9
<i>P. denitrificans</i> DSM 1407		9
<i>P. denitrificans</i> DSM 1408		9
<i>P. denitrificans</i> IFO 12442		6

^a Abbreviations for culture collections: ATCC, American Type Culture Collection, Rockville, Md.; CCM, Czechoslovak Collection of Microorganisms, J. E. Purkyně University, Brno, Czechoslovakia; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; FERM, Fermentation Research Institute, Agency of Industrial Science and Technology, Tukuba, Japan; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; NCIB, National Collection of Industrial Bacteria, Aberdeen, United Kingdom; NRRL, Agricultural Research Service Culture Collection, Northern Regional Research Center, Peoria, Ill.

gen sulfide (TSI medium adjusted to pH 9.0) were not observed. Hydrolysis of gelatin and starch was not observed. (PYG medium adjusted to pH 9.0 was used as a basal medium.) Production of ammonia in peptone water adjusted to pH 9.0 was positive.

Denitrification on PYG agar containing 0.3% (wt/vol) KNO₃ and adjusted to pH 9.0 and denitrification on medium B in which (NH₄)₂SO₄ was replaced with 0.3% (wt/vol) KNO₃ were negative. Litmus milk was not changed. All of the strains grew at the expense of L-arabinose, D-xylose, D-glucose, D-mannose, D-fructose, galactose, D-sorbitol, D-mannitol, inositol, glycerol, succinic acid, citric acid, acetic acid, ethanol, and methanol, but did not grow at the expense of maltose, sucrose, lactose, trehalose, soluble starch, formic acid, dimethylamine, trimethylamine, methane, and hydrogen. Most strains grew at the expense of monomethylamine, but two strains did not, as shown in Table 3. Biotin was required. Ammonia, nitrate, urea, and peptone were utilized by all of the strains as nitrogen sources. Urease, oxidase, and catalase were produced. Growth was observed between pH 7.0 and 9.5, but not below pH 6.5 and above pH 10.0. The optimum cultural pH was pH 8.0 to 9.0. All of the strains grew at 30°C, but did not grow at 37°C. All of the strains grew weakly in the presence of 3% sodium chloride.

Phenotypic characteristics of *P. denitrificans*. Phenotypic characteristics of *P. denitrificans* strains are shown in Tables 2 through 4. All of the strains were gram-negative, nonspore-forming, nonmotile, coccoid (diameter, 0.5 to 0.9 µm) or short rod-shaped (length, 0.9 to 2.0 µm) organisms. Granules of poly-β-hydroxybutyric acid accumulated in the cells.

Colonies were white to light yellow on PYG medium and medium B. Growth in nutrient broth and peptone water was abundant. A water-soluble fluorescent pigment was not produced on King A and King B media. Nitrate was reduced to nitrite. The methyl red test and Voges-Proskauer test were negative. Indole and hydrogen sulfide were not produced. Hydrolysis of gelatin and starch was not observed. Ammonia was produced. Denitrification was performed. Litmus milk was not changed. Table 4 shows the oxidative production of acid from sugars. Acid was not produced fermentatively. All of the strains grew at the expense of D-glucose, D-mannose, D-fructose, trehalose, D-sorbitol, mannitol, inositol, succinic acid, acetic acid, ethanol, and hydrogen, but did not grow at the expense of D-xylose, lactose, soluble starch, formic acid, dimethylamine, trimethylamine, and methane. Most strains grew at the expense of L-arabinose, galactose, maltose, sucrose, glycerol, citric acid, methanol, and monomethylamine, but some strains did not, as shown in Table 3. Vitamins and amino acids were not required. Ammonia and peptone were utilized by all of the strains as nitrogen sources. Most strains utilized nitrate and urea as nitrogen sources, but some strains did not, as shown in Table 2. Urease, oxidase, and catalase were produced. Growth was observed between pH 6.0 and 8.0, but not at pH 5.0 and 9.0. The optimum cultural pH was between pH 6.5 and 7.5. All of the strains grew at 30 and 37°C, but did not grow at 47°C. Some strains grew at 42°C. Growth did not occur in the presence of 3% (wt/vol) NaCl.

DNA base composition. DNA base compositions are shown in Table 5. The DNA base compositions of the group 10 methanol-utilizing bacteria ranged from 64 to 66 mol% G+C,

TABLE 2. Biochemical and physiological characteristics of the group 10 methanol-utilizing bacteria and *P. denitrificans* strains

Strain	Growth in nutrient broth	Growth in peptone water	Nitrate reduction	Denitrification	Production of ammonia	Requirement for biotin	Utilization of nitrate	Utilization of urea	Growth in the presence of:			Growth at:						
									0 NaCl	3% (wt/vol) NaCl	6% (wt/vol) NaCl	pH 6	pH 7	pH 8	pH 9	30°C	37°C	42°C
Isolate TK 1001	w ^a	—	w	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1002	w	w	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1003	w	w	w	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1004	w	w	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1005	w	w	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1006	w	—	w	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1007	w	—	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1008	w	w	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1009	w	w	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1010	w	—	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1011	w	w	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1012	w	w	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1013	w	—	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1014	w	—	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
Isolate TK 1015 ^T	w	w	+	—	+	+	+	+	+	w	—	—	w	+	+	+	—	—
<i>P. denitrificans</i> ATCC 17441 ^T	+	+	+	+	+	—	w	+	+	—	—	+	+	+	—	+	+	—
<i>P. denitrificans</i> ATCC 13543	+	+	+	+	+	—	+	+	+	—	—	+	+	+	—	+	+	—
<i>P. denitrificans</i> IFO 13301	+	+	+	+	+	—	+	+	+	—	—	+	+	+	—	+	+	—
<i>P. denitrificans</i> NCIB 9722	+	+	+	+	+	—	+	+	+	—	—	+	+	+	—	+	+	—
<i>P. denitrificans</i> DSM 1403	+	+	+	+	+	—	+	+	+	—	—	+	+	+	—	+	+	—
<i>P. denitrificans</i> DSM 1404	+	+	+	+	+	—	+	—	+	—	—	+	+	+	—	+	+	—
<i>P. denitrificans</i> DSM 1405	+	+	+	+	+	—	—	—	+	—	—	+	+	+	—	+	+	—
<i>P. denitrificans</i> DSM 1406	+	+	+	+	+	—	+	+	+	—	—	+	+	+	—	+	+	—
<i>P. denitrificans</i> DSM 1407	+	+	+	+	+	—	—	—	+	—	—	+	+	+	—	+	+	—
<i>P. denitrificans</i> DSM 1408	+	+	+	+	+	—	+	+	+	—	—	+	+	+	—	+	+	—
<i>P. denitrificans</i> IFO 12442	+	+	+	+	+	—	+	—	+	—	—	+	+	+	—	+	+	—

^a +, Positive; —, negative; w, weakly positive.

and the DNA base compositions of *P. denitrificans* strains ranged from 65 to 68 mol% G+C.

DNA-DNA homologies. The levels of DNA-DNA homology among the group 10 methanol-utilizing bacteria and *P. denitrificans* strains indicate a clear separation of these bacteria (Table 6). Furthermore, three strains of group 10 methanol-utilizing bacteria showed high levels of similarity to each other, as did three strains of *P. denitrificans*. The DNAs of the group 10 methanol-utilizing bacteria hybridized to the *P. denitrificans* strain DNAs at values of 30 to 60%.

DISCUSSION

The group 10 methanol-utilizing bacteria grew at pH 7.0 to 9.5, and this characteristic is unique in the methanol-utilizing bacteria. They shared almost the same phenotypic characteristics, as well as the same cellular fatty acid composition (16), hydroxy fatty acid composition (16), ubiquinone system (15), and DNA base composition. The strains which we studied showed high levels of similarity to each other in DNA-DNA hybridizations. Therefore, these bacteria fall into a single taxon. These bacteria resembled *P. denitrificans* in morphological characteristics and chemotaxonomic characteristics. Furthermore, these bacteria exhibited less than 44% DNA-DNA homology with *P. denitrificans* strains. On the other hand, the type strain of *P. denitrificans* exhibited less than 58% DNA-DNA homology with the group 10 isolates. The discrepancy in DNA-DNA homology results between the group 10 bacteria and *P. denitrificans* is not clear. Therefore, the group 10 alkaliphilic facultatively methylophilic bacteria seem to be members of the genus *Paracoccus*. We propose a new species, *P. alcaliphilus*, for the

group 10 bacteria. The type strain of *P. alcaliphilus* is strain TK 1015. The minimal characteristics for differentiating *P. alcaliphilus* from *P. denitrificans* are shown in Table 7.

P. denitrificans strains share almost the same phenotypic characteristics, as well as the same cellular fatty acid composition (16), hydroxy fatty acid composition (16), ubiquinone system (15), and DNA base composition. Utilization of methanol was different among the strains, and five strains (DSM 1403, DSM 1404, DSM 1405, DSM 1407, and DSM 1408) did not utilize methanol, as shown by Nokhal and Schlegel (9). Auling et al. (1) reported three clusters, the strain S^T (= ATCC 17441^T) group, the strain M (= DSM 413 = IFO 13301) group, and the strain N4 (= DSM 1404) group, based on a DNA-DNA hybridization study. However, in this study three strains (strains ATCC 17441^T, DSM 1404, and DSM 1405) showed high levels of similarity in DNA-DNA hybridization experiments. Therefore, these group 8 bacteria fall into a single taxon, *P. denitrificans*, as reported by Nokhal and Schlegel (9).

Description of *Paracoccus alcaliphilus* Urakami, Tamaoka, Suzuki, and Komagata sp. nov. *Paracoccus alcaliphilus* (al.cal.i.phil'us. M.L. *alcali*, Eng. alkali from the Arabic *al*, the end, and *qaliy*, soda ash; Gr.adj.*phila*, loving; M.L. adj. *alcaliphilus*, with an affinity for alkalinity [i.e., alkaline media]). Coccoid cells (diameter, 0.5 to 0.9 µm) or short rods (length, 0.9 to 2.0 µm) occur singly, in pairs, or in clusters. Colonies are white to light yellow. Gram-negative. Nonmotile. Granules of poly-β-hydroxybutyric acid accumulate in the cells. Growth in nutrient broth is very poor, but growth in nutrient broth adjusted to pH 9.0 is abundant. Growth in peptone water is poor or nonexistent (Table 2), but growth in

TABLE 3. Utilization of carbon compounds by the group 10 methanol-utilizing bacteria and *P. denitrificans* strains

Strain	Utilization of:																
	L-Arabinose	D-Xylose	D-Galactose	Maltose	Sucrose	Lactose	Trehalose	Inositol	Glycerol	Soluble starch	Succinic acid	Citric acid	Acetic acid	Ethanol	Methanol	Monomethylamine	Hydrogen
Isolate TK 1001	+	+	+	-	-	-	-	+	+	-	+	+	+	+	+	+	-
Isolate TK 1002	+	+	+	-	-	-	-	+	+	-	+	+	w	+	+	+	-
Isolate TK 1003	+	+	+	-	-	-	-	+	w	-	+	+	w	+	+	+	-
Isolate TK 1004	+	+	+	-	-	-	-	+	w	-	+	+	w	+	+	+	-
Isolate TK 1005	+	+	+	-	-	-	-	+	w	-	+	+	w	+	+	+	-
Isolate TK 1006	+	+	+	-	-	-	-	+	w	-	+	+	w	+	+	-	-
Isolate TK 1007	+	+	+	-	-	-	-	+	w	-	+	+	w	+	+	+	-
Isolate TK 1008	+	+	+	-	-	-	-	+	w	-	+	+	+	w	+	-	-
Isolate TK 1009	+	+	+	-	-	-	-	+	w	-	+	+	w	+	+	+	-
Isolate TK 1010	+	+	+	-	-	-	-	+	w	-	+	+	w	+	+	+	-
Isolate TK 1011	+	+	+	-	-	-	-	+	w	-	+	+	+	+	+	+	-
Isolate TK 1012	+	+	+	-	-	-	-	+	w	-	+	+	+	+	+	+	-
Isolate TK 1013	+	+	+	-	-	-	-	+	w	-	+	+	w	+	+	+	-
Isolate TK 1014	+	+	+	-	-	-	-	+	w	-	+	+	w	w	+	+	-
Isolate TK 1015 ^T	+	+	+	-	-	-	-	+	w	-	+	+	+	+	+	+	-
<i>P. denitrificans</i> ATCC 17441 ^T	-	-	-	+	+	-	+	+	+	-	+	-	+	+	+	-	+
<i>P. denitrificans</i> ATCC 13543	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
<i>P. denitrificans</i> IFO 13301	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
<i>P. denitrificans</i> NCIB 9722	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
<i>P. denitrificans</i> DSM 1403	+	-	+	+	+	-	+	+	-	-	+	+	+	+	-	+	+
<i>P. denitrificans</i> DSM 1404	+	-	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+
<i>P. denitrificans</i> DSM 1405	+	-	+	+	+	-	+	+	+	-	+	+	+	+	-	+	+
<i>P. denitrificans</i> DSM 1406	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
<i>P. denitrificans</i> DSM 1407	+	-	+	+	-	-	+	+	+	-	+	+	+	+	-	+	+
<i>P. denitrificans</i> DSM 1408	+	-	+	-	-	-	+	+	+	-	+	-	+	+	-	+	+
<i>P. denitrificans</i> IFO 12442	+	-	+	+	+	-	+	+	+	-	+	-	+	+	+	+	+

^a +, Positive; -, negative; w, weakly positive.

peptone water adjusted to pH 9.0 is abundant. A water-soluble fluorescent pigment is not produced. Nitrate is 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. Denitrification is not performed. Litmus milk is not changed. L-Arabinose, D-xylose, D-glucose, D-mannose, D-fructose,

D-galactose, D-sorbitol, D-mannitol, inositol, glycerol, succinic acid, citric acid, acetic acid, ethanol, and methanol are utilized, but maltose, sucrose, lactose, trehalose, soluble starch, formic acid, dimethylamine, trimethylamine, methane, and hydrogen are not utilized. Utilization of monomethylamine differs among the strains. Biotin is required as an essential supplement. Ammonia, nitrate, urea, and peptone

TABLE 4. Oxidative production of acid from sugars by *P. denitrificans* strains

<i>P. denitrificans</i> strain	Oxidation of:														
	L-Arabinose	D-Xylose	D-Glucose	D-Mannose	D-Fructose	Galactose	Maltose	Sucrose	Lactose	Trehalose	D-Sorbitol	D-Mannitol	Inositol	Glycerol	Soluble starch
ATCC 17441 ^T	- ^a	-	+	+(w)	+	-	+	+	-	+	+	+	+	+	-
ATCC 13543	+	-	+	+(w)	+	+	+	+	-	+	+	+	+	+	-
IFO 13301	+	-	+	+(w)	+	+	+	+	-	+	+	+	+(w)	+	-
NCIB 9722	+	-	-	-	-	+	+(w)	+(w)	-	-	-	-	-	-	-
DSM 1403	+(w)	-	-	-	-	-	-	-	-	-	-	+(w)	-	-	-
DSM 1404	+	-	-	-	+(w)	-	-	+(w)	-	-	-	+(w)	-	-	-
DSM 1405	+	-	-	-	-	-	+	+(w)	-	-	-	+(w)	-	-	-
DSM 1406	+	-	-	-	-	-	-	-	-	-	-	+(w)	+	-	-
DSM 1407	+	-	+	+	+	+	+	+	-	-	+	+	+	+	-
DSM 1408	+	-	+(w)	+	+	-	-	-	-	-	+	+(w)	+	-	-
IFO 12442	+	-	+	+(w)	+	+	+	+	-	+	+	+	-	+	-

^a +, Positive; -, negative; +(w), weak.

TABLE 5. DNA base compositions of the group 10 methanol-utilizing bacteria and *P. denitrificans* strains

Strain	G+C content (mol%)	
	This study	Another study
Isolate TK 1001	64.1	
Isolate TK 1002	64.0	
Isolate TK 1003	64.6	
Isolate TK 1004	64.8	
Isolate TK 1005	64.5	
Isolate TK 1006	64.6	
Isolate TK 1007	64.6	
Isolate TK 1008	64.7	
Isolate TK 1009	64.8	
Isolate TK 1010	64.2	
Isolate TK 1011	64.4	
Isolate TK 1012	64.9	
Isolate TK 1013	65.5	
Isolate TK 1014	64.8	
Isolate TK 1015 ^T	64.4	
<i>P. denitrificans</i> ATCC 17441 ^T	66.9	66.3 ^a
<i>P. denitrificans</i> ATCC 13543		66.6 ^b
<i>P. denitrificans</i> IFO 13301		67.4 ^c
<i>P. denitrificans</i> NCIB 9722		67.0 ^d
<i>P. denitrificans</i> DSM 1404	67.4	65.9 ^b
<i>P. denitrificans</i> DSM 1405	67.2	

^a Data from reference 4.^b Data from reference 18.^c Data from reference 5.^d Data from reference 2.

are utilized as nitrogen sources. Growth is observed between pH 7.0 and 9.5; no growth occurs below pH 6.5 and above pH 10.0. The optimum cultural pH is pH 8.0 to 9.0. Growth is observed at 30°C, but not at 37°C. Growth in the presence of 3% sodium chloride is weak. Urease, oxidase, and catalase are produced. Aerobic. Metabolism is strictly respiratory and not fermentative. The DNA base composition ranges from 64 to 66 mol% G+C. The major cellular fatty acid is straight-chain unsaturated C_{18:1} acid. The major hydroxy fatty acids are 3-hydroxy C_{10:0} acid and 3-hydroxy C_{14:0} acid. The major quinone is ubiquinone Q-10, with Q-9 and Q-11 as minor components.

Variations in phenotypic characteristics, DNA base composition, and DNA-DNA homology in the species are shown in Tables 2, 3, 5, and 6.

The type strain is strain TK 1015.

Description of the type strain. Strain TK 1015^T has all of the characteristics given above for the species. In addition, this strain utilizes monomethylamine. Its DNA base composition is 64.4 mol% G+C. This strain was isolated from soil by T. Urakami (isolation number 0-100). The type strain has

TABLE 6. DNA-DNA homologies among the group 10 methanol-utilizing bacteria and *P. denitrificans* strains

Strain	% DNA-DNA homology with:	
	Strain TK 1015 ^T	Strain ATCC 17441 ^T
Isolate TK 1015 ^T	100	58
Isolate TK 1001	81	57
Isolate TK 1008	61	36
<i>P. denitrificans</i> ATCC 17441 ^T	34	100
<i>P. denitrificans</i> DSM 1404	31	77
<i>P. denitrificans</i> DSM 1405	44	87

TABLE 7. Differential characteristics of *P. alcaliphilus* and *P. denitrificans*

Species	Denitrification	Requirement for biotin	Growth at:		Utilization of:			G+C content (mol%)
			pH 6	pH 9	D-Xylose	Trehalose	Hydrogen	
<i>P. alcaliphilus</i>	— ^a	+	—	+	+	—	—	64–66
<i>P. denitrificans</i>	+	—	+	—	—	+	+	65–68

^a +, Positive; —, negative.

been deposited in the Japan Collection of Microorganisms, Institute of Physical and Chemical Research, as strain JCM 7364.

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