Protomonas, a New Genus of Facultatively Methylotrophic Bacteria

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A new genus and species of facultatively methylotrophic bacteria are described. These bacteria are polarly flagellated, gram-negative, nonsporeforming, rod-shaped organisms that occur singly and in pairs. Carotenoid pigment and bacteriochlorophyll are formed in the cells. The deoxyribonucleic acid base composition is 65 to 67 mol% guanine plus cytosine. The cellular fatty acids consist of a large amount of straight-chain unsaturated C_{18:1} acid and small amounts of straight-chain saturated C_{16:1} acid, C_{19:0} cyclopropane acid, and 3-OH-C_{14:0} hydroxy acid. The major ubiquinone is Q-10. Ubiquinones Q-8, Q-9, and Q-11 are present as minor components. A new genus, *Protomonas*, is proposed to include this group of methylotrophic bacteria. The type species of the genus *Protomonas* is *Protomonas extorquens* comb. nov., with type strain TK 0001 (=DSM 1337 = NCIB 9399).

Previously (44, 45), we described the separation of gram-negative methanol-utilizing bacteria on the basis of morphological characteristics, assimilation of carbon compounds, cellular fatty acid composition, ubiquinone system, and electrophoretic characteristics of enzymes. The group 2 bacteria that we described are facultatively methylotrophic, nonsporeforming, pink-pigmented, polarly flagellated, gram-negative, rod-shaped organisms that have a Q-10 ubiquinone system and a cellular fatty acid composition consisting of a large amount of C_{18:1} acid (type B). "*Protaminobacter ruber*" (11), "*Pseudomonas extorquens*" (3, 25), "*Pseudomonas rosea*" (44, 45; D. G. MacLennan, J. C. Ousby, T. R. Owen, and D. C. Steer, U.K. patent 1,370,892, October 1974), *Pseudomonas* sp. strain ATCC 21438 (44, 45; B. L. Dasinger, A. Clamen, and L. A. Naslund, U.K. patent 1,326,582, August 1973), *Pseudomonas* sp. strain AM1 (35), *Pseudomonas* sp. strain M27 (1), *Pseudomonas* sp. strains 1 and 135 (36), strains 453 and 617 (28), strain 3A2 (10), and isolates V-26, V-157, V-172, BP-3, BP-10, BP-22, and BP-41 (44, 45) are included in this group. "*Pseudomonas extorquens*" NCIB 9399 is a typical strain in this group.

Taxonomic studies of this group have not clarified its status, and the strains may be heterogeneous. Stocks and McCleskey (41) found that "*Protaminobacter ruber*" den Dooren de Jong and *Pseudomonas* sp. strain AM1 of Peel and Quayle were nearly identical to "*Vibrio extorquens*" (Bassalik) Bhat and Barker on the basis of morphological and physiological characteristics. Leadbetter (30) reported that organisms able to use single-carbon organic compounds as well as multicarbon organic compounds required further study. Kouno and Ozaki (28) divided the methanol-utilizing bacteria into seven major groups. These authors included the facultatively methylotrophic, nonsporeforming, pink-pigmented, polarly flagellated, gram-negative, rod-shaped organisms in their group 3. Recently, Green and Bousfield (14) studied gram-negative facultatively methylotrophic bacteria, but did not describe the taxonomic position of such bacteria.

In this study we characterized the facultatively methylotrophic, nonsporeforming, pink-pigmented, polarly flagellated, gram-negative bacteria and established a new genus, *Protomonas*, for these organisms.

MATERIALS AND METHODS

Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; ICPB, International Collection of Phytopathogenic Bacteria, University of California, Davis; IFO, Institute for Fermentation, Osaka, Japan; NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland; NCTC, National Collection of Type Cultures, London, United Kingdom; DNA, deoxyribonucleic acid; G+C, guanine plus cytosine. The abbreviations used for ubiquinones indicate the number of isoprene units in a side chain (e.g., Q-8, Q-9, Q-10, etc.). The abbreviations used for menaquinones are in the following form: MK-*n*(H_m), with *n* indicating the number of isoprene units in the side chain and *m* indicating the number of hydrogen atoms saturating the isoprenoid chain [e.g., MK-9(H₂)].

Bacterial strains. The strains which we studied are listed below. In addition to the bacteria described previously (44, 45), bacteria closely related to this group were included in this study. In the following list, names which do not appear on the Approved Lists of Bacterial Names (40) are enclosed in quotation marks. "*Protaminobacter ruber* subsp. *machidanus*" TK 0006, TK 0007, TK 0008, and TK 0009 were isolated as methanol-utilizing bacteria by Abe and Kobata (U.S. patent 3,764,476, October 1973). *Pseudomonas radiosa* TK 0035 and TK 0036 were isolated as radiation-resistant strains by Ito and Iizuka (21), and assimilation of methanol was mentioned in the original report. *Mycoplana rubra* TK 0002 was isolated by Rhodes (6), *Pseudomonas mesophilica* TK 0034 was isolated from healthy green leaves of *Lolium perenne* by Austin and Goodfellow (2), *Pseudomonas rhodos* TK 0010 was isolated as a star-forming bacterium by Heumann (15), *Pseudomonas* sp. strain TK 0037 was isolated as a mercury-resistant strain by Tonomura et al. (43), and "*Thiobacillus rubellus*" TK 0038 was isolated as a sulfur-oxidizing bacterium by Mizoguchi et al. (32), but assimilation of methanol by these bacteria was not mentioned in the original reports. "*Protaminobacter alboflavus*" TK 0046 was used as a control strain; this strain does not utilize methanol. Cultures were maintained on yeast extract-peptone agar containing 0.25% yeast extract, 0.25% peptone, 0.25% NaCl, and 1.5% agar; this medium was adjusted to pH 7.0. The following strains were studied: "*Mycoplana*

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TABLE 1. Morphological and physiological characteristics of the group 2 bacteria

Strain	Gram stain	Flag- ella- tion	Cell dimensions (μ m)		Color of colonies	Pro- duction of PHB ^a	Fluorescent pigment (King A and B media)	Nitrate reduc- tion	Methyl red test	Voges- Proskauer test	Pro- duction of indole	Pro- duction of H ₂ S (TSI agar)	Hydroly- sis of gelatin	Hydroly- sis of starch	Ur- case	Oxi- dase	Cata- lase
			Width	Length													
" <i>Mycoplasma rubra</i> " TK 0002	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
" <i>Protaminobacter ruber</i> " TK 0003	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
" <i>Pseudomonas extorquens</i> " TK 0001 ^a	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
<i>Pseudomonas</i> sp. strain TK 0004	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
<i>Pseudomonas</i> sp. strain TK 0005	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0006	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0007	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0008	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0009	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
<i>Pseudomonas rhodos</i> TK 0010	-	Polar	0.9	3.0	Pink	+	-	+	-	-	-	-	-	-	+	+	+
" <i>Pseudomonas rosea</i> " TK 0025	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
" <i>Pseudomonas rosea</i> " TK 0023	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
" <i>Pseudomonas rosea</i> " TK 0016	-	Polar	0.9	3.0	Pink	+	-	+(w) ^b	-	-	-	-	-	-	+	+	+
<i>Pseudomonas</i> sp. strain TK 0027	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
<i>Pseudomonas</i> sp. strain TK 0028	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
<i>Pseudomonas</i> sp. strain TK 0029	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
Strain TK 0030	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
Isolate TK 0031	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
Isolate TK 0032	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
Isolate TK 0033	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
<i>Pseudomonas mesophila</i> TK 0034	-	Polar	0.9	3.0	Pink	+	-	+	-	-	-	-	-	-	+	+	+
<i>Pseudomonas radiata</i> TK 0035	-	Polar	0.9	3.0	Pink	+	-	+	-	-	-	-	-	-	+	+	+
<i>Pseudomonas radiata</i> TK 0036	-	Polar	0.9	3.0	Pink	+	-	+	-	-	-	-	-	-	+	+	+
<i>Pseudomonas</i> sp. strain TK 0037	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
" <i>Thiobacillus rubellus</i> " TK 0038	-	Polar	0.9	3.0	Pink	+	-	-	-	-	-	-	-	-	+	+	+
Strain TK 0039	-	Polar	0.9	3.0	Pink	+	-	+	-	-	-	-	-	-	+	+	+
Strain TK 0040	-	Polar	0.9	3.0	Pink	+	-	+	-	-	-	-	-	-	+	+	+
Isolate TK 0042	-	Polar	0.9	3.0	Pink	+	-	+	-	-	-	-	-	-	+	+	+
Isolate TK 0043	-	Polar	0.9	3.0	Pink	+	-	+	-	-	-	-	-	-	+	+	+
Isolate TK 0044	-	Polar	0.9	3.0	Pink	+	-	+	-	-	-	-	-	-	+	+	+
Isolate TK 0045	-	Polar	0.9	3.0	Pink	+	-	+	-	-	-	-	-	-	+	+	+
Isolate TK 0041	-	Polar	0.9	3.0	Pink	+	-	+(w)	-	-	-	-	-	-	+	+	+
" <i>Protaminobacter alboblavus</i> " TK 0046	+	Polar	0.6	3.0	White	+	-	+(w)	-	-	-	-	-	-	+	+	+
					yellow			+									

^a PHB. Poly- β -hydroxybutyrate.
^b w, Weak.

TABLE 2. Morphological and physiological characteristics of the group 2 bacteria

Strain	Produc- tion of ammonia	Requirement for calcium pantothenate as a stimulant	Tolerance to 3% NaCl	Assimi- lation of ammonia	Assimi- lation of nitrate	Assimi- lation of urea	Growth pH					Growth temp (°C)				
							5	6	7	8	9	10	30	37	42	47
"Mycoplana rubra" TK 0002	-	+	-	+	+	+	-	+	+	+	-	+	+	-	-	-
"Protaminobacter ruber" TK 0003	-	-	-	+	+	+	-	+	+	+	-	+	+	-	-	-
"Pseudomonas extorquens" TK 0001 ^T	-	-	-	+	+	+	-	+	+	+	-	+	+	-	-	-
Pseudomonas sp. strain TK 0004	-	-	-	+	+	+	-	+	+	+	-	+	+	-	-	-
Pseudomonas sp. strain TK 0005	-	-	-	+	+	+	-	+	+	+	-	+	+	-	-	-
"Protaminobacter ruber subsp. machidanus" TK 0006	-	-	-	+	+	+	-	+	+	+	+(w) ^a	+	+	+	-	-
"Protaminobacter ruber subsp. machidanus" TK 0007	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-	-
"Protaminobacter ruber subsp. machidanus" TK 0008	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-	-
"Protaminobacter ruber subsp. machidanus" TK 0009	-	-	-	+	+	+	-	+	+	+	+(w)	+	+	+	-	-
Pseudomonas rhodos TK 0010	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-	-
"Pseudomonas rosea" TK 0025	-	-	-	+	+	+	-	+	+	+	-	+	+	+	+(w)	-
"Pseudomonas rosea" TK 0023	-	-	-	+	+	+	-	+	+	+	-	+	+	+	+(w)	-
"Pseudomonas rosea" TK 0016	-	-	-	+	+	+	-	+	+	+	+(w)	+	+	+	+(w)	-
Pseudomonas sp. strain TK 0027	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Pseudomonas sp. strain TK 0028	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Pseudomonas sp. strain TK 0029	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Strain TK 0030	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Isolate TK 0031	-	-	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Isolate TK 0032	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Isolate TK 0033	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Pseudomonas mesophilica TK 0034	-	+	-	+	+	+	-	+	+	+(w)	-	+	+	+	-	-
Pseudomonas radiora TK 0035	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Pseudomonas radiora TK 0036	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Pseudomonas sp. strain TK 0037	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
"Thiobacillus rubellus" TK 0038	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Strain TK 0039	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Strain TK 0040	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Isolate TK 0042	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Isolate TK 0043	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Isolate TK 0044	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Isolate TK 0045	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-
Isolate TK 0041	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-

^a w, Weak.

TABLE 3. Oxidation of sugars by the group 2 bacteria

Strain	Oxidation of:												
	L-Arab- inose	D-Arab- inose	D-Xy- lose	D-Glu- cose	D-Man- nose	D-Fruc- tose	Galac- tose	Malt- ose	Su- crose	Treha- lose	D-Sor- bitol	Glyc- erol	Ri- bose
" <i>Protaminobacter ruber</i> " TK 0003	—	+(w) ^a	—	—	—	—	—	—	—	—	—	+	+
" <i>Pseudomonas extorquens</i> " TK 0001 ^T	+	+(w)	—	—	—	—	—	—	—	—	—	+	+
<i>Pseudomonas</i> sp. strain TK 0004	+	+	—	—	—	—	—	—	—	—	—	+	+
<i>Pseudomonas</i> sp. strain TK 0005	+(w)	+	—	—	—	—	—	—	—	—	—	+	+
<i>Pseudomonas rhodos</i> TK 0010	+(w)	+	—	—	—	+	—	—	—	—	—	+	+
" <i>Pseudomonas rosea</i> " TK 0025	+	+	—	—	—	+	—	—	—	—	—	+	+
" <i>Pseudomonas rosea</i> " TK 0023	+	+	—	—	—	+	—	—	—	—	—	+	+
" <i>Pseudomonas rosea</i> " TK 0016	—	+	—	—	—	+	—	—	—	—	—	+	+
<i>Pseudomonas</i> sp. strain TK 0027	+	+	—	—	—	+	—	—	—	—	—	+	+
<i>Pseudomonas</i> sp. strain TK 0028	+	+	—	—	—	+	—	—	—	—	—	+	+
<i>Pseudomonas</i> sp. strain TK 0029	+	+	—	—	—	+	—	—	—	—	—	+	+
Strain TK 0030	—	+	—	—	—	+	—	—	—	—	—	+	+
Isolate TK 0031	+	+	—	—	—	+	—	—	—	—	—	+	+
Isolate TK 0032	+	+	—	—	—	+	—	—	—	—	—	+	+
Isolate TK 0033	+	+	—	—	—	+	—	—	—	—	—	+	+
Strain TK 0039	+	+	—	—	—	—	+	—	—	—	—	+	—
Strain TK 0040	+	+	—	—	—	—	+	—	—	—	—	+	—
Isolate TK 0042	—	+	—	—	—	—	—	—	—	—	—	—	—
Isolate TK 0043	+	+	—	—	—	—	+	—	—	—	—	—	—
Isolate TK 0044	—	+	—	—	—	—	—	—	—	—	—	—	—
Isolate TK 0045	—	+	—	—	—	—	—	—	—	—	—	—	—
Isolate TK 0041	—	+	—	—	—	—	—	—	—	—	—	+	—

^a w, Weak.

rubra" TK 0002 (= NCIB 10409) (6); "*Protaminobacter ruber*" TK 0003 (= ATCC 8457 = DSM 1340 = IAM 1081 = IFO 3708 = NCIB 2879) (11); "*Protaminobacter ruber* subsp. *machidanus*" TK 0006 (= ATCC 21611), TK 0007 (= ATCC 21612), TK 0008 (= ATCC 21613), and TK 0009 (= ATCC 21614) (S. Abe and M. Kobata, U.S. patent 3,764,476, October 1973); "*Pseudomonas extorquens*" TK 0001 (= DSM 1337 = NCIB 9399) (3, 25); *Pseudomonas mesophilica* TK 0034^T (= ATCC 29983^T = ICPB 4095^T = Austin and Goodfellow A47^T) (T = type strain) (2); *Pseudomonas radiata* TK 0035^T (= ATCC 27329^T = IAM 12099^T) and TK 0036 (= IAM 12098) (21); *Pseudomonas rhodos* TK 0010^T (= ATCC 14821^T) (15); "*Pseudomonas rosea*" TK 0011 (= NCIB 10597), TK 0012 (= NCIB 10598), TK 0013 (= NCIB 10599), TK 0014 (= NCIB 10600), TK 0017 (= NCIB 10601), TK 0015 (= NCIB 10602), TK 0018 (= NCIB 10603), TK 0025 (= NCIB 10604), TK 0026 (= NCIB 10605), TK 0019 (= NCIB 10606), TK 0020 (= NCIB 10607), TK 0021 (= NCIB 10608), TK 0022 (= NCIB 10609), TK 0023 (= NCIB 10610), TK 0016 (= NCIB 10611), and TK 0024 (= NCIB 10612), (44, 45; MacLennan et al., U.K. patent 1,370,892); *Pseudomonas* sp. strain TK 0004 (= ATCC 14718 = DSM 1338 = NCIB 9133 = Peel and Qualye AM1) (35); *Pseudomonas* sp. strain TK 0005 (= DSM 1339 = NCIB 9686 = Anthony and Zatman M27) (1); *Pseudomonas* sp. strain TK 0037 (= Tonomura et al. K-62) (43); *Pseudomonas* sp. strains TK 0027 (= Rock et al. 1) and TK 0028 (= Rock et al. 135) (36); *Pseudomonas* sp. strain TK 0029 (= ATCC 21438) (44, 45; B. L. Dasinger, A. Clamen, and L. A. Naslund, U.K. patent 1,326,582, August 1973); "*Thiobacillus rubellus*" TK 0038 (= Mizoguchi et al. KT-7) (32); strains TK 0030 (= Kouno no. 453) and TK 0039 (= Kouno no. 617) (28); strain TK 0040 (= Colby and Zatman 3A2) (10); isolates TK 0031 (= Urakami and Komagata V-23), TK 0041 (= Urakami and Komagata V-26), TK 0032 (= Urakami and Komagata V-157), TK 0033 (= Urakami and Komagata V-

172), TK 0042 (= Urakami and Komagata BP-3), TK 0043 (= Urakami and Komagata BP-11), TK 0044 (= Urakami and Komagata BP-22), and TK 0045 (= Urakami and Komagata BP-41) (44, 45); and "*Protaminobacter albobiflavus*" TK 0046 (= ATCC 8458 = IAM 1040 = IFO 3707 = NCIB 8167 = NCTC 2875) (11).

Identification method. Yeast extract-peptone agar and medium B containing methanol were used for preculture and basal media. Medium B contained 3.0 g of (NH₄)₂SO₄, 1.4 g of KH₂PO₄, 3.0 g of Na₂HPO₄, 0.2 g of MgSO₄ · 7H₂O, 30 mg of ferric citrate, 30 mg of CaCl₂ · 2H₂O, 5.0 mg of MnCl₂ · 4H₂O, 5.0 mg of ZnSO₄ · 7H₂O, 0.5 mg of CuSO₄ · 5H₂O, 0.2 g of yeast extract, 1.0 ml of a vitamin solution, 10 ml of CH₃OH, and 1,000 ml of distilled water; the pH of this medium was adjusted to 7.1. The vitamin solution used contained 2 mg of biotin, 400 mg of calcium pantothenate, 400 mg of pyridoxine hydrochloride, 400 mg of thiamine hydrochloride, 200 mg of *p*-aminobenzoic acid, 2 mg of folic acid, 2 g of inositol, 400 mg of nicotinic acid, 200 mg of riboflavin, and 1,000 ml of distilled water. Cell form, Gram reaction, motility, and flagellar morphology were determined by using cells grown on yeast extract-peptone agar and medium B agar at 30°C. Gram staining was carried out by the Hucker-Conn modification (16). Motility was observed by using the hanging drop method, and flagellar morphology was determined by the staining method of Toda (42), using an electron microscope and a preparation with negatively stained 2% phosphotungstic acid (pH 7.0). Granules of poly-β-hydroxybutyrate in cells cultivated in medium B containing 0.5% (wt/vol) DL-β-hydroxy-*n*-butyric acid (sodium salt) were stained by the method of Burdon (8). Production of fluorescent pigment was tested on King A and B media. Nitrate reduction was tested in medium B containing 0.1% KNO₃ and in peptone water containing 0.1% KNO₃ after 1, 3, and 7 days of incubation. Voges-Proskauer and methyl red reactions were tested in glucose phosphate-

FIG. 1. Negatively stained cells showing single polar flagella. (A) "*Pseudomonas extorquens*" TK 0001^T, (B) Isolate TK 0044, Bar = 1 μ m.

peptone broth (19) at 30°C. Production of indole was determined with Kovacs reagent in 1% tryptone broth (catalog no. 0123; Difco Laboratories, Detroit, Mich.). Production of hydrogen sulfide was tested on TSI agar for 2 weeks. Hydrolysis of gelatin was observed in stab cultures in yeast extract-peptone medium containing 12% gelatin after 4 weeks of incubation at 20 and 30°C. Hydrolysis of starch was determined by using an iodine solution on yeast extract-peptone agar containing 0.2% soluble starch after 1, 3, and 7 days of incubation. Production of ammonia was tested in peptone water with Nessler's reagent. For the oxidative-fermentative test, OF basal medium (catalog no. 0688; Difco) (17) and PYP medium (46) were used as the basal media. A total of 13 sugars were sterilized by filtration, and each was

added at a concentration of 0.5%. The GasPak anaerobic system (BBL Microbiology Systems, Cockeysville, Md.) was used for anaerobic cultures. Nutritional requirements were determined by using a basal medium without yeast extract and the vitamin solution in medium B. Utilization of nitrogen compounds was determined in liquid basal medium B, and (NH₄)SO₄ was replaced by other nitrogen compounds. Urease activity was observed on Christensen medium (9) for 1 week. The oxidase test was performed with cytochrome oxidase test paper (Nissui Seiyaku, Tokyo, Japan). Catalase activity was detected by the production of bubbles in a 3% hydrogen peroxide solution. Cultural temperature and cultural pH were tested in medium B. Tolerance to sodium chloride was tested in medium B containing

FIG. 2. Photomicrograph of isolate TK 0045 cells showing poly- β -hydroxybutyrate stained with Sudan black B.

3% NaCl. Utilization of carbon compounds was determined in liquid basal medium B without methanol after 3 weeks of cultivation. Succinic acid, citric acid, acetic acid, and methylamine were added at concentrations of 0.15%. A total of 17 carbon compounds were added at concentrations of 0.5%. Assimilation of methane was tested in an atmosphere containing CH_4 , O_2 , and CO_2 (5:4:1).

DNA base composition. Cells cultivated in medium B broth at 30°C for 2 to 3 days with shaking were used for the DNA analysis. DNA was extracted by the method of Saito and Miura (37), and DNA base composition (G+C content) was calculated from the thermal denaturation temperature by using the formula of Marmur and Doty (31).

Cellular fatty acid composition. Cells cultivated in medium B broth at 30°C for 2 to 3 days with shaking were used for the cellular fatty acid analysis. Cellular fatty acid composition was determined as described previously (44).

Quinone system and quinone homologs. Cells cultivated in medium B broth at 30°C for 2 to 3 days with shaking were used for the quinone analysis. Quinone systems were determined as described previously (44). Mass spectrometry (Shimadzu model LKB 9000 single-focusing mass spectrometer) was used to confirm the quinones. Quinone homologs were analyzed by using a Shimadzu model LC-2F high-performance liquid chromatograph and a column (4.4 by 400 mm) of Nucleosil 5C₁₈ (M. Nagel). The solvent used was methanol-*n*-hexane (4:1, vol/vol). The flow rate was 0.7 ml/min. Detection was with a Shimadzu model SPD-1 spectrophotometer at 274 nm.

Pigments. Cells were cultivated in medium B broth at 30°C for 3 days; these cells were illuminated for the first 24 h and then kept in the dark for the following 48 h. The pigments were extracted with 90% isopropanol from whole cells harvested by centrifugation. Carotenoid pigment was purified by the method of Sato et al. (39), and bacteriochlorophyll pigment was purified by the method of Sato (38). The visible and ultraviolet absorption spectra of samples were recorded with a Hitachi model 220A spectrophotometer.

RESULTS

Phenotypic characteristics of the group 2 bacteria. The phenotypic characteristics of the group 2 bacteria are shown in Tables 1 through 4.

All of the strains studied were gram-negative, nonspore-forming, rod-shaped organisms measuring 0.8 to 1.2 by 1.5 to 4.0 μm with rounded ends. The cells occurred singly, rarely in pairs, and were motile by means of single polar flagella. The single polar flagella of "*Pseudomonas extorquens*" TK 0001 and isolate TK 0044 are shown in Fig. 1. Figure 2 shows 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 A and B media. Lighter pink colonies appeared frequently, and colorless colonies ap-

FIG. 3. Effect of calcium pantothenate on the growth of isolate TK 0045. O.D._{610 nm}, Optical density at 610 nm.

FIG. 4. Fatty acid patterns of methanol-utilizing bacteria. (A) "*Pseudomonas extorquens*" TK 0001. (B) "*Protaminobacter ruber*" TK 0003. (C) *Pseudomonas rhodos* TK 0010. (D) *Pseudomonas mesophilica* TK 0034. (E) *Pseudomonas radiora* TK 0035. (F) *Pseudomonas* sp. strain TK 0037.

peared rarely. Nitrate was not reduced to nitrite by most strains. Some strains reduced nitrate weakly (Table 1). The methyl red and Voges-Proskauer tests were negative. Indole and hydrogen sulfide (TSI medium) were not produced. Hydrolysis of gelatin and starch was not observed. Ammonia was not produced. Acid was produced oxidatively from sugars, but the sugars which supported acid production differed among the strains. Acid was not produced fermenta-

tively. Vitamins and amino acids were not essential for the growth of any strain, and calcium pantothenate stimulated the growth of almost one-half of the strains (Fig. 3). Ammonia, nitrate, and urea were assimilated by all of the strains as nitrogen sources. Urease, oxidase, and catalase were produced. Good growth was observed between pH 6.0 and 8.0. *Pseudomonas radiora* TK 0035 and *Pseudomonas* sp. strain TK 0037 grew slowly at pH 5.0. "*Pseudomonas rosea*" TK

TABLE 5. Quinone types and G+C contents of the group 2 bacteria

Strain	Quinone type	Ubiquinone homolog (%)				G+C content (mol%)
		Q-8	Q-9	Q-10	Q-11	
" <i>Mycoplana rubra</i> " TK 0002	Q-10	0.3	3.3	94.9	1.5	
" <i>Protaminobacter ruber</i> " TK 0003	Q-10	0.4	3.5	95.3	0.8	65.6
" <i>Pseudomonas extorquens</i> " TK 0001 ^T	Q-10	0.2	4.7	94.0	1.1	65.6
<i>Pseudomonas</i> sp. strain TK 0004	Q-10	0.1	3.9	95.1	0.9	
<i>Pseudomonas</i> sp. strain TK 0005	Q-10	0.3	2.9	95.8	1.0	
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0006	Q-10		3.1	96.4	0.5	
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0007	Q-10	0.3	3.0	95.3	1.4	65.9
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0008	Q-10	0.2	3.5	96.3		
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0009	Q-10	0.2	2.6	96.5	0.7	
<i>Pseudomonas rhodos</i> TK 0010	Q-10	0.2	4.0	95.2	0.6	65.8
" <i>Pseudomonas rosea</i> " TK 0025	Q-10	0.3	2.8	95.2	1.0	
" <i>Pseudomonas rosea</i> " TK 0023	Q-10	0.5	4.0	94.9	0.6	
" <i>Pseudomonas rosea</i> " TK 0016	Q-10	0.2	3.5	95.1	1.2	65.4
<i>Pseudomonas</i> sp. strain TK 0027	Q-10	0.2	3.7	95.1	1.0	
<i>Pseudomonas</i> sp. strain TK 0028	Q-10	0.1	3.0	94.9	2.0	66.1
<i>Pseudomonas</i> sp. strain TK 0029	Q-10	0.3	2.7	95.8	1.2	
Strain TK 0030	Q-10	0.5	3.4	96.1		
Isolate TK 0031	Q-10	0.2	1.9	96.5	1.4	
Isolate TK 0032	Q-10	0.3	2.8	95.9	1.0	65.6
Isolate TK 0033	Q-10		3.5	96.1	0.4	
<i>Pseudomonas mesophilica</i> TK 0034	Q-10	0.1	2.3	96.7	0.9	66.0
<i>Pseudomonas radiora</i> TK 0035	Q-10	0.5	4.7	93.8	1.0	65.8
<i>Pseudomonas radiora</i> TK 0036	Q-10	0.3	4.3	94.1	1.3	
<i>Pseudomonas</i> sp. strain TK 0037	Q-10	0.2	4.9	94.0	0.9	66.2
" <i>Thiobacillus rubellus</i> " TK 0038	Q-10	0.2	3.7	94.7	1.4	65.4
Strain TK 0039	Q-10	0.4	4.0	95.1	0.5	
Strain TK 0040	Q-10	0.3	3.0	95.5	1.2	
Isolate TK 0042	Q-10	0.4	5.6	93.0	1.0	
Isolate TK 0043	Q-10	0.3	4.5	93.8	1.4	
Isolate TK 0044	Q-10	0.3	3.6	94.9	1.2	66.6
Isolate TK 0045	Q-10	0.2	4.3	94.5	1.0	
Isolate TK 0041	Q-10	0.2	2.7	95.4	1.7	
" <i>Protaminobacter alboflavus</i> " TK 0046	MK-9(H ₂)					

0016 and "*Protaminobacter ruber* subsp. *machidans*" TK 0006 and TK 0009 grew slowly at pH 9.0. All of the strains grew at 30°C, but the temperature range for growth differed among the strains. Growth did not occur in the presence of 3% sodium chloride. All of the strains grew at the expense of methanol, glycerol, and succinic acid, but did not grow at the expense of lactose, D-mannitol, inositol, or methane. The strains tested were divided into three subgroups on the basis of assimilation of L-arabinose, D-xylose, D-glucose, D-fructose, galactose, and methylamine. The first subgroup assimilated methylamine, but did not assimilate L-arabinose, D-xylose, D-glucose, D-fructose or galactose. The second subgroup assimilated D-fructose and methylamine, but did not assimilate L-arabinose, D-xylose, D-glucose, or galactose. The third subgroup assimilated L-arabinose, D-xylose, D-glucose, and galactose, but did not assimilate methylamine.

On the other hand, "*Protaminobacter alboflavus*" TK 0046, which was used as a control strain, was a gram-positive, nonsporeforming, irregularly rod-shaped organism measuring 0.5 to 0.7 by 1.5 to 4.0 µm (usually barrel or club shaped). Snapping division produced angular and palisade arrangements of cells. A pink or red water-insoluble pigment was not produced. This strain must belong to the coryneform group.

DNA base composition. DNA base composition was tested by using 12 strains selected from subtypes based on an electrophoretic comparison of enzyme and assimilation of carbon compounds. The DNA base composition of these strains ranged from 65 to 67 mol% G+C (Table 5).

The DNA base composition of *Pseudomonas mesophilica* A47 (65 ± 0.8 mol% G+C) was reported by Austin and Goodfellow (2), that of *Pseudomonas radiora* 0-1 (= IAM 12099) (ca. 65.0 mol% G+C) was reported by Ito and Iizuka (22), and that of "*Thiobacillus rubellus*" KT-7 (65 ± 3 mol% G+C) was reported by Mizoguchi et al. (32). These values were confirmed by us.

Cellular fatty acid composition. All of the strains studied contained a large amount of straight-chain unsaturated C_{18:1} acid (Table 6 and Fig. 4). This cellular acid composition is type B (44). Small amounts of straight-chain saturated C_{16:1} acid, C_{19:0} cyclopropane acid, and 3-OH-C_{14:0} hydroxy acid were also detected. On the other hand, "*Protaminobacter alboflavus*" TK 0046 contained large amounts of straight-chain saturated C_{16:0} and C_{18:0} acids and straight-chain unsaturated C_{16:1} and C_{18:1} acids. Hydroxy acids were not detected (Table 6 and Fig. 5).

Quinone system. All of the strains studied had the Q-10 ubiquinone system, along with minor ubiquinone Q-8, Q-9, and Q-11 components (Table 5). The occurrence of ubiquinones Q-8, Q-9, and Q-11 as minor components in *Pseudomonas* sp. strain AM1, *Pseudomonas* sp. strain M27, and "*Protaminobacter ruber*" has been reported by Natori and Nagasaki (33).

On the other hand, "*Protaminobacter alboflavus*" TK 0046 had the MK-9(H₂) menaquinone system.

Pigments. The crude pigments extracted from all of the strains tested had identical absorption spectra. An absorption maximum at approximately 500 nm indicated carotenoids, and an absorption maximum at ca. 770 nm indicated

TABLE 6. Cellular fatty acid compositions of the group 2 bacteria^a

Strain	Straight-chain acids								Cyclopropane acids		3-OH-C ₁₄ hydroxy acid	Unknown peak X-2
	C _{14:0}	C _{14:1}	C _{16:0}	C _{16:1}	C _{17:0}	C _{18:0}	C _{18:1}	C _{19:0}	C _{17:0}	C _{19:0}		
" <i>Mycoplana rubra</i> " TK 0002			4.0 ^b	6.6	0.8	6.4	76.4	0.6			0.2	
" <i>Protaminobacter ruber</i> " TK 0003			4.0	7.0	0.7	8.3	76.6	1.4	0.3		1.8	
" <i>Pseudomonas extorquens</i> " TK 0001 ^T			4.3	13.1	0.2	2.1	72.5				4.8	
<i>Pseudomonas</i> sp. strain TK 0004			2.1	6.0	1.0	6.7	79.1	2.0	0.5		1.0	
<i>Pseudomonas</i> sp. strain TK 0005			5.4	7.5	1.2	7.5	74.0	1.7	0.5		2.2	
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0006			4.7	1.1	1.8	9.1	81.0				1.7	
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0007			4.7	0.7	0.4	8.9	83.4				1.9	
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0008			4.3	0.9	1.0	8.5	84.0				1.3	
" <i>Protaminobacter ruber</i> subsp. <i>machidanus</i> " TK 0009			3.8	1.5	1.2	9.5	83.0				1.0	
<i>Pseudomonas rhodos</i> TK 0010			3.2	5.2	0.2	7.6	80.0				1.3	
" <i>Pseudomonas rosea</i> " TK 0025			2.9	0.5	1.9	7.4	82.9	1.7	0.4		2.3	
" <i>Pseudomonas rosea</i> " TK 0023			2.2	1.6	1.5	4.0	86.7				4.0	
" <i>Pseudomonas rosea</i> " TK 0016			2.0	1.0	1.5	5.0	85.5	1.6	0.4		3.0	
<i>Pseudomonas</i> sp. strain TK 0027			2.9	0.5	0.4	6.6	86.4				3.2	
<i>Pseudomonas</i> sp. strain TK 0028			3.5	1.2	0.9	4.9	86.0	1.0			2.5	
<i>Pseudomonas</i> sp. strain TK 0029			3.0	1.5	1.8	4.5	86.0	1.5	0.2		1.5	
Strain TK 0030			2.7	0.4	0.6	10.3	84.6				1.5	
Isolate TK 0031			3.2	2.0	0.6	10.6	80.8				2.2	
Isolate TK 0032			9.0	1.3	0.3	7.8	78.0	1.8			1.8	
Isolate TK 0033			3.3	1.8	0.5	2.3	88.5				3.6	
<i>Pseudomonas mesophilica</i> TK 0034			5.2	2.0	0.4	6.8	84.6				0.9	
<i>Pseudomonas radiora</i> TK 0035			3.1	0.5	0.4	7.9	85.8				0.9	
<i>Pseudomonas radiora</i> TK 0036			5.5	0.7	0.7	10.6	81.6				0.9	
<i>Pseudomonas</i> sp. strain TK 0037			3.8	0.5	0.5	9.8	83.6				1.8	
" <i>Thiobacillus rubellus</i> " TK 0038			3.0	0.6	0.3	6.7	87.9				0.8	
Strain TK 0039			5.1	1.1	0.2	5.7	86.8				1.1	
Strain TK 0040			3.3	0.2	0.2	8.4	87.1				1.1	
Isolate TK 0042			3.5	1.4	0.6	5.0	88.0				1.5	
Isolate TK 0043			4.2	1.9	0.4	3.1	88.5				1.9	
Isolate TK 0044			4.0	1.7	0.8	4.6	87.5				1.4	
Isolate TK 0045			4.7	1.5	0.5	2.9	89.5				0.9	
Isolate TK 0041			3.0	1.1	0.3	3.9	90.8				1.0	
" <i>Protaminobacter alboflavus</i> " TK 0046	3.1	0.6	24.2	12.1	0.8	12.1	39.3	0.7	0.5	0.2		4.1

^a The retention times of the straight-chain acids relative to the retention time of straight-chain C_{16:0} acid (defined as 1.00) were as follows: C_{14:0}, 0.53; C_{14:1}, 0.62; C_{16:1}, 1.17; C_{17:0}, 1.43; C_{18:0}, 1.91; C_{18:1}, 2.20; C_{19:0}, 2.60. The relative retention times of C_{17:0} and C_{19:0} cyclopropane acids were 1.58 and 3.08, respectively; the relative retention times of 3-OH-C₁₄ hydroxy acid and unknown peak X-2 were 3.17 and 3.54, respectively.

^b Percentage of the acid to the total of acids.

bacteriochlorophyll (Fig. 6). "*Pseudomonas extorquens*" TK 0001 was selected for purification of pigments. Bacteriochlorophyll was not formed in cells cultivated throughout in light, but was formed in cells cultivated throughout in darkness and in cells cultivated under illumination for the first 24 h and then in the dark for the following 48 h. Carotenoid pigment and bacteriochlorophyll were observed in the cells of all of the test strains, but were not detected in the cells of the control strain, "*Protaminobacter alboflavus*" TK 0046.

The formation of carotenoid pigment in *Pseudomonas* sp. strain AM1, *Pseudomonas* sp. strain M27, "*Protaminobacter ruber*," and strains 453 and 617 has been reported by Peel and Quayle (35), Anthony and Zatman (1), Sato et al. (39), and Kouno and Ozaki (28), respectively. Bacteriochlorophyll formation in "*Protaminobacter ruber*" and *Pseudomonas* sp. strain AM1 has been reported by Sato (38), whereas bacteriochlorophyll formation in *Pseudomonas* ra-

diora has been reported by Nishimura et al. (34). These findings were confirmed by us.

DISCUSSION

The 45 strains of facultative methanol-utilizing, pink-pigmented, polarly flagellated, gram-negative bacteria and other related bacteria used in this study shared almost the same phenotypic characteristics, as well as the same cellular fatty acid composition, ubiquinone system, DNA base composition, carotenoid pigment, and bacteriochlorophyll. Furthermore, the serine pathway of methanol metabolism was supported by an electrophoretic comparison of enzymes, as previously described (45). Therefore, these bacteria fall into a single taxon.

These bacteria appear to have some of the characteristics of strains in the genus *Pseudomonas*, and several have been identified as being in this genus, such as "*Pseudomonas extorquens*," "*Pseudomonas rosea*," *Pseudomonas* sp.

strain AM1, and *Pseudomonas* sp. strain M27. However, these methanol-utilizing bacteria are distinguished from typical *Pseudomonas* strains by their cellular fatty acid composition (20), by their ubiquinone systems (47), and by formation of bacteriochlorophyll.

Several facultative methanol-utilizing bacteria have been identified as members of the genus "*Protaminobacter*"; these include "*Protaminobacter ruber*" and "*Protaminobacter ruber* subsp. *machidanus*." The genus "*Protaminobacter*" is not on the Approved Lists of Bacterial Names (40); furthermore, "*Protaminobacter alboflavus*," the type species of this genus, is gram positive and nonmotile and has a menaquinone system. Therefore, allocation of the methanol-utilizing bacteria which we studied to the genus "*Protaminobacter*" would be inappropriate. De Vries and Derx (12) proposed a transfer of "*Protaminobacter rubrum*" den Dooren de Jong to "*Mycoplana rubra*" (den Dooren de Jong) de Vries and Derx in 1953, but the bacteria in group 2 described previously were distinguished from *Mycoplana dimorpha* NCIB 9439, the type strain of the type species of this genus (13), by morphological and physiological characteristics and by formation of a carotenoid pigment and bacteriochlorophyll. Therefore, allocation of the above-mentioned methanol-utilizing bacteria to the genus *Mycoplana* would be inappropriate. In addition, the genus *Mycoplana* is a poorly defined genus, and the taxonomic status of this genus should be clarified.

The genus *Thiobacillus* is heterogeneous in morphological, physiological, and chemotaxonomic characteristics (18, 24, 27). The facultative methanol-utilizing bacteria which we studied resemble *Thiobacillus novellus* in cellular fatty acid composition, ubiquinone system, and DNA base composition, but were distinguished by cell size, motility, assimilation of carbon compounds, and formation of a carotenoid pigment and bacteriochlorophyll (26).

As mentioned above, the facultative methanol-utilizing, pink-pigmented, polarly flagellated, gram-negative bacteria were clearly distinguished from related bacteria by phenotypic and chemotaxonomic characteristics. The facts in this study and the above-mentioned taxonomic considerations led us to establish a new genus for this group of bacteria.

In 1913, Bassalik (4) isolated a rod-shaped bacterium from the excreta of earthworms in forest and garden soils and reported the assimilation of oxalate by this organism. Bassalik named this organism "*Bacillus extorquens*." The name of this organism has since been changed to "*Vibrio extorquens*" by Bhat and Barker (5), "*Pseudomonas extorquens*" by Krassilnikov (29), "*Vibrio extorquens*" by Iyer et al. (23), and "*Flavobacterium extorquens*" by Bassalik et al. (3).

The original isolates of Bassalik (4) perished during World War II, and other authentic cultures on which the description was based have not been available. However, in 1950 Janota (25) reported the isolation of a bacterium which resembled the organisms of Bassalik and proposed the name "*Pseudomonas extorquens*." One of the cultures was deposited at the National Collection of Industrial Bacteria, Aberdeen, Scotland under the name "*Flavobacterium extorquens*" in 1960, and this culture has been maintained as "*Pseudomonas extorquens*" NCIB 9399^T to the present time. Therefore, this strain should have been regarded as the neotype strain of "*Bacillus extorquens*" Bassalik, and the taxonomic position and status of this organism should have been assessed by the appropriate subcommittee of the International Committee for Systematic Bacteriology.

According to *Index Bergeyana* (7), "*Bacillus extorquens*" Bassalik 1913, "*Vibrio extorquens*" (Bassalik) Bhat and

FIG. 5. Fatty acid pattern of "*Protaminobacter alboflavus*" TK 0046.

Barker 1948, "*Pseudomonas extorquens*" (Bassalik) Krassilnikov 1949, and "*Flavobacterium extorquens*" (Bassalik) Bassalik, Janota-Bassalik, and Brisou 1960 are validly published, legitimate names. After considering the phenotypic and chemotaxonomic characteristics and the historical background of these facultative methanol-utilizing, pink-pigmented, polarly flagellated, gram-negative, rod-shaped organisms, we propose to establish a new genus, *Protomonas*, for this group of bacteria, and designate *Protomonas extorquens* as the type species of this genus. The type strain is *Protomonas extorquens* TK 0001.

Description of *Protomonas* Urakami and Komagata gen. nov. *Protomonas*, (Pro.to.mo'nas. Gr.n. *protos* original; Gr.n. *monas* unit, monad; M.L.fem.n. *Protomonas* the original monad) cells are nonsporeforming, 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, rarely in pairs, and are motile by means of single polar flagella. 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.

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 from sugars. Methanol is assimilated; methane is not assimilated. Urease, oxidase, and catalase are produced. Aerobic. Metabolism is strictly respiratory and not fermentative.

DNA base composition ranges from 65 to 67 mol% G+C. The cellular fatty acids are composed of a large amount of straight-chain saturated C_{18:1} acid, and small amounts of straight-chain saturated C_{16:1} acid, C_{19:0} cyclopropane acid, and 3-OH-C_{14:0} hydroxy acid. The major ubiquinone is Q-10.

The type species is *Protomonas extorquens* (Bassalik) Urakami and Komagata comb. nov.

Description of *Protomonas extorquens* (Bassalik) Urakami and Komagata comb. nov. *Protomonas extorquens* [basonym, *Bacillus extorquens* Bassalik 1913, 258; synonyms, *Vibrio extorquens* (Bassalik) Bhat and Barker 1948, *Pseudomonas extorquens* (Bassalik) Krassilnikov 1949, and *Flavobacterium extorquens* (Bassalik) Bassalik, Janota-Bassalik and Brisou 1960] cells are regular rods that are 0.8 to 1.0 by

FIG. 6. Absorption spectrum of the crude pigment extracted with 90% (vol/vol) isopropanol from cells of isolate TK 0044.

1.5 to 4.0 μm and have rounded ends. Cells occur singly, rarely in pairs, and are motile by means of single polar flagella (Fig. 1). Granules of poly- β -hydroxybutyric acid accumulate in the cells. Endospores do not form in the cells. Gram negative.

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. Cultures in peptone broth are turbid, and a ring is formed at the surface. A non-diffusible, nonfluorescent, water-insoluble, pink pigment is produced.

Nitrate is not reduced to nitrite. (Some strains reduce nitrate weakly [Table 1].) 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 from L-arabinose, D-arabinose, glycerol, and ribose oxidatively, but not from D-glucose, D-mannose, D-fructose, maltose, sucrose, trehalose, or sorbitol. (The sugars which support production of acid differ among the strains [Table 3].) Utilizes glycerol, succinic acid, ethanol, acetic acid, methanol, and methylamine as sole carbon sources for energy and growth, but does not utilize L-arabinose, D-xylose, D-glucose, D-mannose, D-fructose, galactose, maltose, sucrose, lactose, trehalose, D-sorbitol, D-mannitol, inositol, soluble starch, citric acid, or methane. (Utilization of L-arabinose, D-xylose, D-glucose, D-mannose, D-fructose, galactose, trehalose, citric acid, acetic acid, ethanol, and methylamine differs among the strains [Table 4].) Vitamins and amino acids are not required for growth. (Some strains require calcium pantothenate as a stimulant [Table 2].) Ammonia, nitrate, and urea are assimilated. Urease, oxidase, and catalase are produced. Aerobic. Metabolism is strictly respiratory and not fermentative.

Good growth occurs between pH 6.0 and 8.0. Good growth occurs at 30°C but not at 37°C. (The temperature for

growth differs among the strains [Table 2].) Growth does not occur in media containing 3% sodium chloride.

The DNA base composition is 65.6 mol% G+C (variable from 65 to 67 mol% G+C [Table 5]). The cellular fatty acids are composed of a large amount of straight-chain unsaturated $\text{C}_{18:1}$ acid, and small amounts of straight-chain unsaturated $\text{C}_{16:1}$ acid, $\text{C}_{19:0}$ cyclopropane acid, and 3-OH- $\text{C}_{14:0}$ hydroxy acid. The major ubiquinone is Q-10. Ubiquinones Q-8, Q-9, and Q-11 appear as minor components. The red or pink pigment is a carotenoid pigment, and the absorption maxima are 465, 495, and 525 nm (Fig. 6). Bacteriochlorophyll is formed under specific cultural conditions (Fig. 6). Methanol is utilized by the serine pathway with formaldehyde incorporation, and the complete tricarboxylic acid cycle is functional (1).

The type strain is TK 0001 (= DSM 1337 = NCIB 9399), which was isolated from Polish soil by Janota in 1950. This strain was deposited in the National Collection of Industrial Bacteria, Aberdeen, Scotland, as "*Flavobacterium extorquens*" by C. Bassalik in 1960.

According to this study and other investigations, *Pseudomonas rhodos* Heuman 1962 (15), *Pseudomonas radora* Ito and Iizuka 1971 (21), and *Pseudomonas mesophilica* Austin and Goodfellow (2) should be identified as strains of *Protomonas extorquens*.

The bacteria belonging to *Protomonas extorquens* differ from each other with respect to oxidative production of acids from sugars (Table 3), assimilation of carbon compounds (Table 4), requirements for vitamins (Table 2), pH for growth (Table 2), temperature for growth (Table 2), and electrophoretic patterns of enzymes (45). This species was divided into three subgroups based on assimilation of carbon compounds (44), and this grouping agreed well with the grouping based on an electrophoretic comparison of enzymes (45). Subgroups 1 and 2 correspond to cluster 10, and subgroup 3 corresponds to cluster 11 of Green and Bousfield (14). However, these differences were recognized as intraspecific.

Therefore, we propose biovars 1, 2, and 3 of *Protomonas extorquens*. *Protomonas extorquens* TK 0001^T (= DSM 1337^T = NCIB 9399^T) is a representative strain of biovar 1, *Protomonas extorquens* TK 0010 (= ATCC 14821) is a representative strain of biovar 2, and *Protomonas extorquens* TK 0035 (= ATCC 27329 = IAM 12099) is a representative strain of biovar 3. Biovar 1 contains TK 0001 to TK 0005; biovar 2 contains TK 0006 to TK 0033; and biovar 3 contains TK 0034 to TK 0045.

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LITERATURE CITED

- Anthony, C., and L. J. Zatman. 1964. Microbial oxidation of methanol. I. Isolation and properties of *Pseudomonas* sp. M 27. *Biochem. J.* 92:609–614.
- Austin, B., and M. Goodfellow. 1979. *Pseudomonas mesophilica*, a new species of pink bacteria isolated from leaf surfaces. *Int. J. Syst. Bacteriol.* 29:373–378.
- Bassalik, C., L. Janota-Bassalik, and J. Brisou. 1960. Etude sur *Flavobacterium extorquens* (ex. *Pseudomonas extorquens*). *Ann. Inst. Pasteur Paris* 98:165–168.
- Bassalik, K. 1913. Über die Verarbeitung der Oxalsäure durch *Bacillus extorquens* n. sp. *Jahrb. Wiss. Bot.* 53:255–302.
- Bhat, J. V., and H. A. Barker. 1948. Studies on a new oxalate-decomposing bacterium, *Vibrio oxaliticus*. *J. Bacteriol.* 55:359–368.
- Bousfield, I. J., and S. D. Graham (ed.). 1975. The National Collection of Industrial Bacteria catalogue of strains, 3rd ed. Her Majesty's Stationery Office, London.
- Buchanan, R. E., J. G. Holt, and E. F. Lessel, Jr. (ed.). 1966. Index Bergeyana. The Williams & Wilkins Co., Baltimore.
- Burdon, K. L. 1946. Fatty material in bacteria and fungi revealed by staining dried, fixed slide preparations. *J. Bacteriol.* 52:665–678.
- Christensen, W. B. 1946. Urea decomposition as a means of differentiating *Proteus* and *Paracolon* cultures from each other. *J. Bacteriol.* 52:461–466.
- Colby, J., and L. J. Zatman. 1973. Trimethylamine metabolism in obligate and facultative methylotrophs. *Biochem. J.* 132:101–112.
- Den Dooren de Jong, L. E. 1927. Über protaminophage Bakterien. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2* 71:193–232.
- De Vries, J. T., and H. G. Derx. 1953. On the occurrence of *Mycoplana rubra* and its identity with *Protaminobacter rubrum*. *Ann. Bogor.* 1:53–60.
- Gray, P. H. H., and H. G. Thornton. 1928. Soil bacteria that decompose certain aromatic compounds. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2* 73:74–96.
- Green, P. N., and I. J. Bousfield. 1982. A taxonomic study of some gram-negative facultatively methylotrophic bacteria. *J. Gen. Microbiol.* 128:623–638.
- Heumann, W. 1962. Die Methodik der Kreuzung sternbildender Bakterien. *Biol. Zentralbl.* 81:341–354.
- Hucker, G. J., and H. J. Conn. 1923. Method of Gram staining. *Technical Bulletin* 93. New York State Agricultural Experiment Station, Ithaca.
- Hugh, R., and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *J. Bacteriol.* 66:24–26.
- Hutchinson, M., K. I. Johnstone, and D. White. 1965. The taxonomy of certain thiobacilli. *J. Gen. Microbiol.* 41:357–366.
- Iizuka, H., and K. Komagata. 1963. An attempt at grouping the genus *Pseudomonas*. *J. Gen. Appl. Microbiol.* 9:73–82.
- Ikemoto, S., H. Kuraishi, K. Komagata, R. Azuma, T. Suto, and H. Murooka. 1978. Cellular fatty acid composition in *Pseudomonas* species. *J. Gen. Appl. Microbiol.* 24:199–213.
- Ito, H., and H. Iizuka. 1971. Taxonomic studies on a radio-resistant *Pseudomonas*. XII. Studies on the microorganisms of cereal grain. *Agric. Biol. Chem.* 35:1566–1571.
- Ito, H., and H. Iizuka. 1980. Characterization of radiation-resistant species of *Pseudomonas radiora* and pattern of catalase activity. *Agric. Biol. Chem.* 44:1315–1320.
- Iyer, V. N., S. R. Khambata, and J. V. Bhat. 1960. The characteristics and questionable taxonomic position of the oxalate-decomposing bacterium, *Vibrio extorquens*. *Proc. Indian Acad. Sci. Sect. B* 51:157–163.
- Jackson, J. F., D. J. W. Moriarty, and D. J. D. Nicholas. 1968. Deoxyribonucleic acid base composition and taxonomy of thiobacilli and some nitrifying bacteria. *J. Gen. Microbiol.* 53:53–60.
- Janota, L. 1950. Przebieg zuzywania krasu szczawowego przez *Pseudomonas extorquens* Bassalik, w zaleznosci od poczatkowej liczby komorek. *Med. Dosw. Mikrobiol.* 2:131–132.
- Katayama-Fujimura, Y., and H. Kuraishi. 1980. Characterization of *Thiobacillus novellus* and its thiosulfate oxidation. *J. Gen. Appl. Microbiol.* 26:357–367.
- Katayama-Fujimura, Y., N. Tsuzaki, and H. Kuraishi. 1982. Ubiquinone, fatty acid and DNA base composition determination as a guide to the taxonomy of the genus *Thiobacillus*. *J. Gen. Microbiol.* 128:1599–1611.
- Kouno, K., and A. Ozaki. 1975. Distribution and identification of methanol-utilizing bacteria, p. 11–21. In *Proceedings of the International Symposium on Microbial Growth on C₁ Compounds*. Society of Fermentation Technology, Osaka.
- Krassilnikov, N. A. 1949. Guide to the bacteria and actinomycetes. Akademii Nauk S.S.S.R., Moscow. (In Russian.)
- Leadbetter, E. R. 1974. Family IV. *Methylomonadaceae* Leadbetter 1974, p. 267–269. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed., The Williams & Wilkins Co., Baltimore.
- Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* 5:109–118.
- Mizoguchi, T., T. Sato, and T. Okabe. 1976. New sulfur-oxidizing bacteria capable of growing heterotrophically, *Thiobacillus rubellus* nov. sp. and *Thiobacillus delicatus* nov. sp. *J. Ferment. Technol.* 54:181–191.
- Natori, Y., and T. Nagasaki. 1980. Occurrence of coenzyme Q₁₂ and coenzyme Q₁₃ in facultative methanol-oxidizing bacteria. *Agric. Biol. Chem.* 44:2105–2110.
- Nishimura, Y., M. Shimadzu, and H. Iizuka. 1981. Bacteriochlorophyll formation in radiation-resistant *Pseudomonas radiora*. *J. Gen. Appl. Microbiol.* 27:427–430.
- Peel, D., and J. R. Quayle. 1961. Microbial growth on C₁ compounds. I. Isolation and characterization of *Pseudomonas* AM 1. *Biochem. J.* 81:465–469.
- Rock, J. S., I. Goldberg, A. Ben-Bassat, and R. I. Mateles. 1976. Isolation and characterization of two methanol-utilizing bacteria. *Agric. Biol. Chem.* 40:2129–2135.
- Saito, H., and K. Miura. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta* 72:619–629.
- Sato, K. 1978. Bacteriochlorophyll formation by facultative methylotrophs, *Protaminobacter ruber* and *Pseudomonas* AM 1. *FEBS Lett.* 85:207–210.
- Sato, K., T. Mizutani, M. Hiraoka, and S. Shimizu. 1982. Carotenoid containing sugar moiety from a facultative methylotroph, *Protaminobacter ruber*. *J. Ferment. Technol.* 60:111–115.
- Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.). 1980. Approved lists of bacterial names. *Int. J. Syst. Bacteriol.* 30:225–420.

41. Stocks, P. K., and C. S. McCleskey. 1964. Identity of the pink-pigmented methanol-oxidizing bacteria as *Vibrio extorquens*. J. Bacteriol. **88**:1065-1070.
42. Toda, T. 1923. A staining method of bacterial flagella. Nihon Iji Shinpo **283**:113.
43. Tonomura, K., T. Nakagami, F. Futai, and K. Maeda. 1968. Studies on the action of mercury-resistant microorganisms and the binding of mercurials to the cells. J. Ferment. Technol. **46**:506-512.
44. Urakami, T., and K. Komagata. 1979. Cellular fatty acid composition and coenzyme Q system in gram negative methanol-utilizing bacteria. J. Gen. Appl. Microbiol. **25**:343-360.
45. Urakami, T., and K. Komagata. 1981. Electrophoretic comparison of enzymes in the gram negative methanol-utilizing bacteria. J. Gen. Appl. Microbiol. **27**:381-403.
46. Yabuuchi, E., E. Tanimura, A. Ohyama, I. Yano, and A. Yamamoto. 1979. *Flavobacterium devorans* ATCC 10829: a strain of *Pseudomonas paucimobilis*. J. Gen. Appl. Microbiol. **25**:95-107.
47. Yamada, Y., H. Takinami-Nakamura, Y. Tahara, H. Oyaizu, and K. Komagata. 1982. The ubiquinone systems in the strains of *Pseudomonas* species. J. Gen. Appl. Microbiol. **28**:7-12.