J. Gen. Appl. Microbiol., **29**, 17–40 (1983)

GROUPING OF *PSEUDOMONAS* SPECIES ON THE BASIS OF CELLULAR FATTY ACID COMPOSITION AND THE QUINONE SYSTEM WITH SPECIAL REFERENCE TO THE EXISTENCE OF 3-HYDROXY FATTY ACIDS

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(Received October 7, 1982)

The cellular fatty acids and the quinone systems were investigated in 46 strains of *Pseudomonas* species including 14 phytopathogenic *Pseudomonas* strains. In a total of 75 strains, including 46 strains in this study and 29 strains reported in previous papers, *Pseudomonas* species showed heterogeneity in fatty acid composition and in the ubiquinone system. They were divided into nine groups according to these characteristics, with special reference to the existence of 3-hydroxy fatty acids. The significance of 3-hydroxy fatty acid composition in the classification of *Pseudomonas* species is discussed.

Interrelation of bacteria has been shown by the indices of deoxyribonucleic acid (DNA)-deoxyribonucleic acid homology (DNA-DNA homology) and ribosomal ribonucleic acid (rRNA)-deoxyribonucleic acid homology (rRNA-DNA homology), and by the similarity of the sequence of ribosomal RNA. However, such parameters are difficult to apply in the classification and routine identification of bacteria. Instead, the chemical constituents of bacterial cells, such as DNA base compositions (G+C contents), cellular fatty acids, quinone systems, and amino acids in the peptidoglycans, have been examined for use in classification and rapid identification.

Previously, IKEMOTO et al. (*1*) reported the cellular fatty acid composition, and YAMADA et al. (*2*) investigated quinone systems in the strains of the genus *Pseudomonas*. Following these studies, the present authors further investigated the cellular fatty acid composition and quinone system in *Pseudomonas* species including phytopathogenic species, and found that the genus showed heterogeneity in 3-hydroxy fatty acid composition.

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Table 1. Bacterial strains studied.

Species and strain designations		Other strain designations[a]							3-OH fatty acids[b]				Quinone[c] system	References
		AJ	ATCC	DSM	IAM	IFO	KM	NCIB	10	12	14	16		
Section I														
P. aeruginosa	KS 0024	2115	7700		1275				+	+	−	−	Q-9	
P. aeruginosa	KS 0025*	2116	10145		1514				+	+	−	−	Q-9	
P. putida	KS 0100							8859					Q-9	
P. fluorescens	KS 0009	2018			1218				+	+	−	−	Q-9	
P. fluorescens	KS 0022	2089							+	+	−	−	Q-9	
P. fluorescens	KS 0112*		13525		12022			9046	+	+	−	−	Q-9	
P. chlororaphis	KS 0015*	2066	9446		1511				+	+	−	−	Q-9	
P. aureofaciens	KS 0004	2001	13986		1001				+	+	−	−	Q-9	
P. stutzeri	KS 0013		9114		1054								Q-9	
P. mendocina	KS 0097							10541					Q-9	
P. alcaligenes	KS 0018	2080	12815						+	+	−	−	Q-9	
P. alcaligenes	KS 0021*	2085	14909					9945	+	+	−	−	Q-9	
P. azotoformans	KS 0034*	2173			1603				+	+	−	−	Q-9	
P. fulva	KS 0029	2125			1587				+	+	−	−	Q-9	
P. fulva	KS 0030	2126											Q-9	
P. nitroreducens	KS 0050*	2282			1439				+	+	−	−	Q-9	
"*P. ovalis*"	KS 0008	2011			1002				+	+	−	−	Q-9	
"*P. ovalis*"	KS 0010	2022			1050				+	+	−	−	Q-9	
P. straminea	KS 0028*	2124							+	+	−	−	Q-9	
P. straminea	KS 0270	3130											Q-9	
P. taetrolens	KS 0017	2071											Q-9	
P. taetrolens	KS 0235	2072				3458							Q-9	
P. taetrolens	KS 0237	2088											Q-9	
P. taetrolens	KS 0238	2315											Q-9	
P. taetrolens	KS 0239	2348											Q-9	
P. taetrolens	KS 0240	2925											Q-9	
"*P. lacunogenes*"	KS 0036	2197			1568				+	+	−	−	Q-9	

Table 1. (continued)

Species and strain designations		Other strain designations[a]							3-OH fatty acids[b]				Quinone[c] system	References
		AJ	ATCC	DSM	IAM	IFO	KM	NCIB	10	12	14	16		
"*P. lacunogenes*"	KS 0037	2198			1579				+	+	−	−	Q-9	
"*P. lacunogenes*"	KS 0222	2201			1601								Q-9	
"*P. ochracea*"	KS 0026	2122											Q-9	
"*P. ochracea*"	KS 0027	2123											Q-9	
P. mucidolens	KS 0038	2213	4685					9394	+	+	−	−	Q-9	
Section II														
P. cepacia	KS 0052	2404	17759				645		−	−	+	+	Q-8	
P. cepacia	KS 0233						627						Q-8	
P. cepacia	KS 0234						644						Q-8	
Section III														
P. testosteroni	KS 0043*	2230	11996					8955	+	−	−	−	Q-8	
P. testosteroni	KS 0048	2270	17409						+	−	−	−	Q-8	
P. acidovorans	KS 0056	3116	15667					9682	+	−	−	−	Q-8	
P. acidovorans	KS 0057*	3117	15668					9681	+	−	−	−	Q-8	
P. flava	KS 0231*			619									Q-8	
P. palleronii	KS 0230*		17724	63									Q-8	
P. pseudoflava	KS 0232*			1034									Q-8	
P. iners	KS 0046*	2265			1419				+	−	−	−	Q-8	
P. iners	KS 0047	2267			1445				+	−	−	−	Q-8	
"*P. cruciviae*"	KS 0005	2002			1048				+	−	−	−	Q-8	
"*P. dacunhae*"	KS 0006	2003			1089				+	−	−	−	Q-8	
"*P. desmolytica*"	KS 0054	3111	15005						+	−	−	−	Q-8	
"*Comamonas terrigenna*"	KS 0020	2083	8461					8193	+	−	−	−	Q-8	
Section IV														
P. maltophilia	KS 0001*	2082	13637					9203	−	+	−	−	Q-8	
P. maltophilia	KS 0002	2220	17806		1554				−	+	−	−	Q-8	
P. maltophilia	KS 0131	2475							−	+	−	−	Q-8	
P. pictorum	KS 0271	2141						9152					Q-8	

Table 1. (continued)

Species and strain designations		Other strain designations[a]							3-OH fatty acids[b]				Quinone[c] system	References
		AJ	ATCC	DSM	IAM	IFO	KM	NCIB	10	12	14	16		
P. vesicularis	KS 0241*		11426		12105								Q-10	
P. diminuta	KS 0016*	2067	11568		1513				−	+	−	−	Q-10	
P. diminuta	KS 0242								−	+	−	−	Q-10	(*1*, *2*)
P. diminuta	KS 0243								−	+	−	−	Q-10	(*1*, *2*)
Others														
P. paucimobilis	KS 0300*						2395		−	−	−	−	Q-10	
P. paucimobilis	KS 0301						2396		−	−	−	−	Q-10	
"*P. extorquens*"	KS 0111							9399	−	−	+	−	Q-10	
"*P. rosea*"	KS 0312							10597					Q-10	
Pseudomonas sp. BP-22													Q-10	(*3*, *4*)

* Type strain.

[a] Abbreviations for culture collections: AJ, Central Research Laboratories, Ajinomoto Co., Kawasaki, Japan; ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; KM, Kansai Medical University, Moriguchi City, Osaka, Japan; and NCIB, National Collection of Industrial Bacteria, Aberdeen, U. K.

[b] Data are cited from references (*1*, *5*, *6*). The numbers, 10, 12, 14, and 16 indicate the number of carbon atoms in the 3-hydroxy fatty acids. +, fatty acid present; −, fatty acid absent.

[c] Data are cited from references (*2*, *3*, *6*).

This paper deals with the cellular fatty acid composition and quinone system of *Pseudomonas* species and discusses the grouping of the genus *Pseudomonas* based on the 3-hydroxy fatty acid composition and ubiquinone system.

MATERIALS AND METHODS

Bacterial strains studied. For this study the fifty eight non-phytopathogenic *Pseudomonas* strains studied in the previous papers (*1*, *2*) were used, as well as three methanol-utilizing bacteria, "*Pseudomonas extorquens*" KS 0111, "*Pseudomonas rosea*" KS 0312, and *Pseudomonas* sp. BP-22 (*3*, *4*), and fourteen phytopathogenic *Pseudomonas* strains. Their sources and designations in other culture collections are listed in Tables 1 and 2. The three methanol-utilizing strains are representatives of the three subgroups of the group 2 reported by URAKAMI and KOMAGATA (*4*). Data for the 3-hydroxy fatty acid compositions and the ubiquinone systems reported previously (*1–3*, *5*, *6*) are listed in Table 1. The species are arranged according to Bergey's Manual (*7*), and those not listed in the Manual are added to the appropriate sections covering the systems of IIZUKA and KOMAGATA (*8*, *9*) and of STANIER et al. (*10*). The identity of the phytopathogenic *Pseudomonas* strains was confirmed by their morphological and phenotypic characteristics. The species names are used according to their designations when they were received from culture collections and individuals. Names which are not on the Approved Lists of Bacterial Names, 1980 (*11*) are enclosed in quotation marks. *Pseudomonas maltophilia* and *Pseudomonas cepacia* are not enclosed in quotation marks because these species were revived by HUGH (*12*) and by PALLERONI and HOLMES (*13*).

Cellular fatty acid composition. Fatty acids were analyzed according to the

Table 2. Phytopathogenic bacterial strains studied.

Species and strain designations	Sources	
P. avenae	KS 0256	NIAS 1024, isolated from *Zea mays*
P. avenae	KS 0257	NIAS 1027, isolated from *Agropyron trichophorum*
P. caryophylli	KS 0250	NIAS 1060, isolated from *Dianthus caryophyllus*
P. gladioli pv. *gladioli*	KS 0258	NIAS 1065, isolated from *Freesia reflacta*
P. solanacearum	KS 0261	NIAS 1067, isolated from *Solanum melongena*
P. solanacearum	KS 0262	NIAS 1069, isolated from *Nicotiana tabacum*
P. syringae pv. *coronafaciens*	KS 0252	NIAS 1061, isolated from *Avena sativa*
P. syringae pv. *coronafaciens*	KS 0253	NIAS 1016, isolated from *Avena sativa*
P. syringae pv. *eriobotryae*	KS 0254	NIAS 1062, isolated from *Eriobotrya japonica*
P. syringae pv. *japonica*	KS 0263	NIAS 1071, isolated from *Triticum aestivum*
P. syringae pv. *japonica*	KS 0264	NIAS 1072, isolated from *Hordeum vulgare*
P. syringae pv. *mori*	KS 0259	NIAS 1020, isolated from *Morus bombycis*
P. syringae pv. *mori*	KS 0260	NIAS 1021, isolated from *Morus bombycis*
P. syringae pv. *tabaci*	KS 0265	NIAS 1073, isolated from *Nicotiana tabacum*

NIAS, National Institute of Agricultural Science, Tsukuba, Ibaraki, Japan. Sources of isolation are cited from the list of cultures in the plant pathology division of NIAS (*46*).

Table 3. Difference in 3-hydroxy fatty acid composition with two fatty acid-liberation methods.

Species and strain designations		A[a]							B						
		3-OH 8:0	3-OH 10:0	3-OH 12:0	3-OH 12:1	3-OH 14:0	3-OH 14:1	3-OH 16:0	3-OH 8:0	3-OH 10:0	3-OH 12:0	3-OH 12:1	3-OH 14:0	3-OH 14:1	3-OH 16:0
Group 1															
P. aeruginosa	KS 0025	0[b]	39	61	0	0	0	0	0	28	72	0	0	0	0
Group 2															
P. cepacia	KS 0052	0	0	0	0	57	0	43	0	0	0	0	31	0	69
Group 3															
P. acidovorans	KS 0057	0	100	0	0	0	0	0	0	100	0	0	0	0	0
P. pseudoflava	KS 0232	0	100	0	0	0	0	0	0	100	0	0	0	0	0
Group 4															
P. diminuta	KS 0016	0	0	89	3	8	0	0	0	0	63	2	35	0	0
P. diminuta	KS 0242	0	0	90	1	9	0	0	0	0	70	0	30	0	0
P. diminuta	KS 0243	0	0	85	1	14	0	0	0	0	56	0	44	0	0
P. vesicularis	KS 0241	0	0	63	3	34	0	0	0	0	71	4	25	0	0
Group 6															
P. paucimobilis	KS 0300	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group 7															
"*P. extorquens*"	KS 0111	0	0	0	0	100	0	0	0	0	0	0	100	0	0
Group 8															
P. palleronii	KS 0230	100	0	0	0	0	0	0	100	0	0	0	0	0	0
Group 9															
P. avenae	KS 0256	0	27	4	4	33	32	0	0	10	4	8	42	36	0

[a] A, liberation by methanolysis with 5% HCl-methanol; B, liberation by hydrolysis with 4 N HCl.

[b] Numbers are the ratio of the 3-hydroxy fatty acids to the total of all 3-hydroxy fatty acids.

Abbreviations of fatty acids are the same as those described for Tables 5 and 6.

method reported previously (*1*, *5*, *14*). Cellular fatty acids were liberated from cells by methanolysis with 5% HCl-methanol, and their composition was determined for 32 non-phytopathogenic strains and 14 phytopathogenic strains. For comparison with the methanolysis method, cellular fatty acids of 13 strains were liberated by hydrolysis with 4 N HCl (ca. 11.7% HCl) at 100° for 5 hr, and extracted with ethyl ether. The ether fraction was evaporated under nitrogen current, and methylated with 5% HCl-methanol. The hydroxy fatty acids were separated by thin layer chromatography (TLC) (*1*). In strains which had a large amount of 2-hydroxy fatty acid, the 2-hydroxy fatty acid fraction was separated from the 3-hydroxy fatty acid fraction by TLC. Unknown hydroxy fatty acids were identified by gas-liquid chromatography-mass spectrometry (GC-MS) by the method described by SUZUKI et al. (*15*).

Quinone system. Quinone systems were determined by the method of YAMADA et al. (*16*).

RESULTS

Effect of hydrolysis of cells with 4 N HCl on 3-hydroxy fatty acid composition

As amide-bound hydroxy fatty acids may be not entirely liberated from cells by methanolysis with 5% HCl-methanol (personal communication from Dr. K. Kawahara, Institute of Applied Microbiology, The University of Tokyo), the effect of liberation by hydrolysis of cells with 4 N HCl was examined. For comparison with earlier results (*1*, *3*, *5*, *6*), the 3-hydroxy fatty acid was composition determined for thirteen strains by the two different liberation procedures. Results are shown in Tables 3 and 4.

Differences in components of the 3-hydroxy fatty acids due to the two liberation methods were not found in all the strains tested, but there was a little difference in the quantity of 3-hydroxy fatty acids. One kind of 3-hydroxy fatty acid occurred in *Pseudomonas acidovorans* KS 0057, *Pseudomonas pseudoflava* KS 0232, "*P. extorquens*" KS 0111, and *Pseudomonas palleronii* KS 0230; two or more kinds occurred in *Pseudomonas aeruginosa* KS 0025, *Pseudomonas cepacia* KS 0052, *Pseudomonas diminuta* KS 0016, KS 0242 and KS 0243, *Pseudomonas vesicularis* KS 0241, *Pseu-*

Table 4. Difference in 3-hydroxy fatty acid composition with two fatty acid liberation methods (*Pseudomonas maltophilia* KS 0001).

	2-OH *i*-11	3-OH *i*-11	3-OH 11:0	3-OH *i*-12	3-OH 12:0	3-OH *i*-13	Unknown (Number of peaks)
A[a]	11[b]	18	2	6	25	28	9 (3)
B	7	12	2	4	39	31	4 (2)

[a] A, liberation by methanolysis with 5% HCl-methanol; B, liberation by hydrolysis with 4N HCl.

[b] Numbers are the ratio of the 3-hydroxy fatty acids to the total of all 3-hydroxy fatty acids. Abbreviations of fatty acids are the same as those described for Tables 5 and 6.

domonas avenae KS 0256, and *P. maltophilia* KS 0001. *Pseudomonas paucimobilis* KS 0300 had no 3-hydroxy fatty acid. Compared with methanolysis with 5% HCl-methanol, hydrolysis with 4 N HCl increased the amount of 3-hydroxy fatty acid with longer carbon length and decreased those with shorter carbon length. This tendency was found in the strains of *P. aeruginosa*, *P. cepacia*, *P. diminuta*, *P. avenae*, and *P. maltophilia*, which had two or more kinds of 3-hydroxy fatty acids. The liberation of 3-hydroxy fatty acids with 4 N HCl seems to be better than with 5% HCl-methanol. However, the former liberation procedure is much more time-consuming than the latter. Therefore, the methanolysis method was employed for detection of the characteristic 3-hydroxy fatty acids.

Cellular fatty acid composition of the strains of non-phytopathogenic Pseudomonas species

The cellular fatty acid composition of 32 non-phytopathogenic *Pseudomonas* strains is shown in Table 5. The fatty acids of *P. maltophilia* KS 0001 and *Pseudomonas pictorum* KS 0241 were composed mainly of *iso*- and *anteiso*-branched-chain fatty acids. The fatty acids of the other 30 strains were mostly even-numbered straight-chain fatty acids of $C_{16:0}$, $C_{16:1}$, and $C_{18:1}$.[1] The 32 strains studied had characteristic 3-hydroxy fatty acid composition. 3-OH $C_{10:0}$ and 3-OH $C_{12:0}$ occurred in *P. aeruginosa* KS 0025, *Pseudomonas fulva* KS 0030, "*Pseudomonas lacunogenes*" KS 0222, *Pseudomonas mendocina* KS 0097, "*Pseudomonas ochracea*" KS 0026 and KS 0027, *Pseudomonas putida* KS 0100, *Pseudomonas straminea* KS 0270, *Paeudomonas stutzeri* KS 0013, and *Pseudomonas taetrolens* KS 0017, KS 0235, KS 0237, KS 0238, KS 0239, and KS 0240. *Pseudomonas cepacia* KS 0052, KS 0233, and KS 0234 contained 3-OH $C_{14:0}$ and 3-OH $C_{16:0}$. *Pseudomonas acidovorans* KS 0057, *Pseudomonas testosteroni* KS 0043, "*Comamonas terrigena*" KS 0020, *Pseudomonas flava* KS 0231, and *Pseudomonas pseudoflava* KS 0232 contained 3-OH $C_{10:0}$. *Pseudomonas diminuta* KS 0016 and *P. vesicularis* KS 0241 contained 3-OH $C_{12:0}$ and 3-OH $C_{14:0}$. *Pseudomonas maltophilia* KS 0001 and *P. pictorum* KS 0271 contained 3-OH $C_{12:0}$, 3-OH *i*-$C_{11:0}$, 3-OH *i*-$C_{12:0}$, and 3-OH *i*-$C_{13:0}$. *Pseudomonas paucimobilis* KS 0300 had no 3-hydroxy fatty acid. *Pseudomonas pallironii* KS 0230 had 3-OH $C_{8:0}$. "*Pseudomonas extorquens*" KS 0111, "*P. rosea*" KS 0312, and *Pseudomonas* sp. BP-22 had 3-OH $C_{14:0}$.

Cellular fatty acid composition in phytopathogenic Pseudomonas species

The even-numbered straight-chain fatty acids $C_{16:0}$, $C_{16:1}$, and $C_{18:1}$ were found as major components in all the phytopathogenic *Pseudomonas* strains, as shown in Table 6. The strains tested had the characteristic 3-hydroxy fatty acid components. *Pseudomonas syringae* pv. *coronafaciens* KS 0252 and KS 0253, *P. syringae* pv.

[1] The abbreviations used for fatty acids are: *i*, *iso*-acid; *a*, *anteiso*-acid; 2-OH or 3-OH, 2- or 3-hydroxy acid; $C_{16:0}$, a straight-chain saturated acid of 16-carbon; $C_{16:0}$, a straight-chain unsaturated acid of 16-carbon with a double bond, e. g., *i*-$C_{15:0}$, 3-OH $C_{10:0}$, etc.

Table 5. Cellular fatty acid composition of the strains of *Pseudomonas* species.

Fatty acids	*P. aeruginosa* KS 0025	*P. fluva* KS 0030	"*P. lacunogenes*" KS 0222	*P. mendocina* KS 0097	"*P. ochracea*" KS 0026	"*P. ochracea*" KS 0027	*P. putida* KS 0100	*P. straminea* KS 0270	*P. stutzeri* KS 0013	*P. taetrolens* KS 0017	*P. taetrolens* KS 0235	*P. taetrolens* KS 0237	*P. taetrolens* KS 0238	*P. taetrolens* KS 0239	*P. taetrolens* KS 0240	*P. cepacia* KS 0052	*P. cepacia* KS 0233	*P. cepacia* KS 0234	*P. acidovorans* KS 0057	*P. testosteroni* KS 0043	"*Comamonas terrigena*" KS 0020	*P. flava* KS 0231	*P. pseudoflava* KS 0232	*P. diminuta* KS 0016	*P. vesicularis* KS 0241	*P. maltophilia* KS 0001	*P. pictorum* KS 0271	*P. paucimobilis* KS 0300	"*P. extorquens*" KS 0111	"*P. rosea*" KS 0312	*Pseudomonas* sp. KS 0313	*P. palleronii* KS 0230
8:0																							1.2	1.6									2.6
12:0	3.4	4.7	7.7	9.5	9.1	10.1	4.7	2.0	7.8	3.5	5.3	3.1	7.2	1.9	7.8	T	T		2.6	2.8	2.4			1.3								0.6
14:0	0.5	0.9	T	1.3	0.9	0.8	T	0.8	1.2	T	1.4	0.5	0.7	0.7	0.9	4.5	4.1	5.5	0.7	T	3.1	3.0	3.8	3.2	1.3	4.1	1.7	T	3.4			T
15:0	T	0.6	T	1.7	2.1	1.4	T	T	T	T	T	T	T	0.5	0.6	0.5	T		0.7	T	2.3			2.7	4.0	1.0	5.2					
16:0	28.0	32.4	20.5	18.6	19.9	17.7	34.5	38.3	19.4	33.7	35.1	29.6	35.8	32.9	38.3	19.1	26.5	25.2	30.7	28.2	32.1	23.9	19.3	45.4	18.2	15.4	5.2	5.7	3.4	1.7	6.3	20.3
16:1	6.0	11.1	21.2	22.2	20.8	22.4	20.9	5.5	26.4	10.5	18.4	27.1	5.8	40.0	11.6	5.0	24.0	3.4	26.4	20.5	36.2	44.3	57.8	2.2	5.7	13.7	12.7	2.9	7.1	T	1.9	41.1
17:0	T		T	T			T	0.8	T	1.0	0.6		1.1			1.1	T	0.9	1.2	1.3	1.5	0.7	0.5	2.6	2.7			0.5		0.6	T	0.5
18:0	0.8	1.0	0.5	0.5	0.6	0.7	1.2	1.1	1.0	1.2	0.9	1.5	0.9	0.8	0.7	1.0	1.1	0.8	0.9	T		1.3	0.7	1.2	1.3	0.6	T		4.4	6.2	3.8	T
18:1	35.6	22.9	39.7	34.3	33.3	35.1	11.1	15.7	32.5	7.3	7.1	15.4	3.0	10.3	4.7	26.5	34.4	8.0	18.3	21.2	12.1	20.6	8.3	11.6	56.0	1.8	0.6	63.0	80.8	88.4	85.6	21.2
19:0		5.0	T	T	T	T	6.6	7.3	1.2	9.5	6.7	3.8	10.9	T	9.0	5.3	T	11.4	4.2	4.1	2.1		0.9									3.5
Δ17	2.7	4.9	T	2.2	1.6	1.4	6.8	8.0	0.9	9.1	6.3	3.9	11.1	0.5	9.4	9.6	T	8.3	7.7	10.7	4.2	T	1.2	0.7	6.3		0.5					2.5
Δ19	1.4	0.6	T		T	T	1.9	0.8	1.0	1.7	T	0.8	2.4		0.8	5.7		3.9		1.2				15.9	0.5							
3-OH 8:0																																5.3
3-OH 10:0	2.6	3.1	3.2	4.5	5.2	4.4	3.5	4.8	2.8	5.5	5.1	3.2	5.1	2.8	4.5				3.0	3.8	3.8	3.1	4.1									
3-OH 12:0	4.1	3.1	2.9	2.5	3.0	3.3	2.5	2.9	2.6	3.8	3.4	2.7	3.7	2.9	3.3									6.0	1.7	1.9	0.6					
3-OH 12:1																								T								
3-OH 14:0																2.9	4.4	10.0						0.5	0.9				2.3	2.2	1.3	
3-OH 16:0																1.6	2.6	5.6														
2-OH 12:0	8.2	4.9	2.1	0.7	1.4	1.2	3.6	6.9		5.2	4.1	4.7	1.7	6.3	1.7																	
2-OH 14:0																				0.6								20.2				
2-OH 16:0		0.7					1.1	1.1	T	1.4	1.2	0.6	1.9		1.4	0.8		1.5		2.9												
2-OH 18:0																T				1.0												
2-OH 18:1																0.6																
2-OH Δ19																1.1																
i-11																2.1										2.7	2.4					
i-13																										T	T					
i-14																										0.6	16.4					
i-15																								2.3	0.9	25.1	19.4					
a-15																										14.6	3.9					
i-16																										1.0	16.1					
i-17:1																										4.0	9.0					
3-OH *i*-11																										1.5	1.6					
3-OH *i*-12																										0.6	2.9					
3-OH *i*-13																										2.7	1.4					
2-OH *i*-11																										1.4						

[a] The abbreviations used for fatty acids are: *i*, *iso*-acid; Δ, cyclopropane acid; 2-OH or 3-OH, 2- or 3-hydroxy acid; 16:0, a straight-chain saturated acid of 16-carbon; 16:1, a straight-chain unsaturated acid of 16-carbon with a double bond.

[b] Numbers refer to the ratio of the fatty acids to total acids. T, Acid present less than 0.5%.

eriobotryae KS 0254, *P. syringae* pv. *japonica* KS 0263 and KS 0264, *P. syringae* pv. *mori* KS 0259 and KS 0260, and *P. syringae* pv. *tabaci* KS 0265 had 3-OH $C_{10:0}$ and 3-OH $C_{12:0}$. *Pseudomonas caryophylli* KS 0250, *Pseudomonas gladioli* pv. *gladioli* KS 0258, and *Pseudomonas solanacearum* KS 0261 and KS 0262 had 3-OH $C_{14:0}$ and 3-OH $C_{16:0}$. *Pseudomonas avenae* KS 0256 and KS 0257 had 3-OH $C_{10:0}$, 3-OH $C_{12:0}$, 3-OH $C_{12:1}$, 3-OH $C_{14:0}$, and 3-OH $C_{14:1}$.

3-Hydroxy fatty acid composition of the strains in Pseudomonas diminuta

The presence of 3-OH $C_{14:0}$ in lipopolysaccharide of *P. diminuta* NCTC 8545 was shown by WILKINSON et al. (*17*). However, IKEMOTO et al. (*1*), KALTENBACH et al. (*18*), and MOSS and DEES (*19*) did not report 3-OH $C_{14:0}$ in the strains of *P. diminuta*. According to our results, Tables 3 and 5, only a small amount of 3-OH $C_{14:0}$ (0.5% of total fatty acids and 8–14% of total 3-hydroxy fatty acids) was liberated from cells by methanolysis with 5% HCl-methanol. However, a relatively large amount of 3-OH $C_{14:0}$ (30–44% of total 3-hydroxy fatty acids) was liberated from cells by hydrolysis with 4 N HCl. Therefore, the strains in *P. diminuta* are characterized by the presence of 3-OH $C_{12:0}$ and 3-OH $C_{14:0}$, as shown in Fig. 4.

3-Hydroxy fatty acid composition of the strains in Pseudomonas maltophilia

IKEMOTO et al. (*6*) reported the cellular fatty acid composition of the 14 strains of *P. maltophilia* including *P. maltophilia* KS 0001, the type strain, and found 3-OH $C_{12:0}$ and four unknown hydroxy fatty acids (A-OH, B-OH, C-OH, and D-OH) in these strains. The hydroxy fatty acids of *P. maltophilia* KS 0001 were identified by their retention time in gas-liquid chromatography and by GC-MS, and it was confirmed that this strain had 2-OH *i*-$C_{11:0}$, 3-OH *i*-$C_{11:0}$, 3-OH $C_{12:0}$, and 3-OH *i*-$C_{13:0}$ as major hydroxy fatty acids (Fig. 5). Therefore, the unknown hydroxy fatty acids reported by IKEMOTO et al. (*6*), A-OH, B-OH, C-OH, and D-OH, are considered to be 2-OH *i*-$C_{11:0}$, 3-OH *i*-$C_{11:0}$, 3-OH *i*-$C_{12:0}$, and 3-OH *i*-$C_{13:0}$, respectively. MOSS et al. (*20*) and MOSS and DEES (*21*) reported the occurrence of the same kinds of hydroxy fatty acids in *P. maltophilia* as those identified in this study. The strains of *P. maltophilia* characteristically have many kinds of 3-hydroxy fatty acids such as 3-OH *i*-$C_{11:0}$, 3-OH $C_{12:0}$, and 3-OH *i*-$C_{13:0}$.

Quinone system in Pseudomonas species

All the strains of the phytopathogenic *Pseudomonas* species had ubiquinone systems (Q-8[2] or Q-9), as shown in Table 6. *Pseudomonas syringae* pv. *coronafaciens* KS 0252 and KS 0253, *P. syringae* pv. *eriobotryae* KS 0254, *P. syringae* pv. *japonica* KS 0263 and KS 0264, *P. syringae* pv. *mori* KS 0259 and KS 0260, and *P. syringae* pv. *tabaci* KS 0265 had Q-9. *Pseudomonas avenae* KS 0256 and KS 0257, *P.*

[2] The abbreviations used for ubiquinone are: Q-*n* with *n* denoting a specified number of isoprene units in a side chain.

Table 6. Cellular fatty acid composition and quinone

Fatty acids	*P. avenae*		*P. caryophylli* KS 0250	*P. gladioli* pv. *gladioli* KS 0258	*P. solanacearum*	
	KS 0256	KS 0257			KS 0261	KS 0262
12:0	2.7	2.2	T	0.7	3.4	T
14:0	2.6	2.3	3.4	3.5	5.3	4.4
15:0	T	T	T	T	0.5	T
16:0	29.4	31.0	19.7	28.6	28.5	19.3
16:1	37.8	42.3	19.5	13.7	19.4	27.5
17:0			0.5	0.9	1.7	1.0
18:0			1.6	2.9	0.6	0.7
18:1	9.9	14.5	42.2	17.2	25.0	28.0
19:0	1.3	T	1.7	5.4	1.1	1.2
Δ17	1.3	T	0.8	8.9	1.8	2.9
Δ19	T			3.0		0.9
3-OH 10:0	2.9	2.6				
3-OH 12:0	0.6	T				
3-OH 12:1	0.8	0.6				
3-OH 14:0	4.4	1.2	5.8	3.6	8.5	4.1
3-OH 14:1	4.8	1.9				
3-OH 16:0			2.4	2.0	T	T
2-OH 12:0						
2-OH 16:0			0.7			
i-11						
i-13						
i-14						
i-15						
a-15						
i-16						
i-17:1						
3-OH *i*-11						
3-OH *i*-12						
3-OH *i*-13						
2-OH *i*-11						
Quinone system	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8

[a] The abbreviations used for fatty acids are: Δ, cyclopropane acid; 2-OH or 3-OH, 2- or 3-
rated acid of 16-carbon with a double bond.

[b] Numbers refer to the ratio of the fatty acids to total fatty acids. T, Acid present less than

[c] Abbreviations used for ubiquinone: Q-8, ubiquinone with eight isoprene units in a side

caryophylli KS 0250, *P. gladioli* pv. *gladioli* KS 0258, and *P. solanacearum* KS 0261 and KS 0262 had Q-8. The quinone systems of the non-phytopathogenic *Pseudomonas* species described in the previous papers (*2*, *3*, *6*) are listed in Table 1.

DISCUSSION

Grouping of Pseudomonas species based on 3-hydroxy fatty acid composition and quinone system

systems of phytopathogenic pseudomonads.

P. syringae pv. *cornafaciens*		*P. syringae* pv. *eriobotryae*	*P. syringae* pv. *japonica*		*P. syringae* pv. *mori*		*P. syringae* pv. *tabaci*
KS 0252	KS 0253	KS 0254	KS 0263	KS 0264	KS 0259	KS 0260	KS 0265
4.1	3.7	4.2	4.7	4.8	3.6	4.2	3.7
0.5	T	T	T	T	T	T	T
T	T	T	T	T	T	T	
27.0	26.7	24.3	30.4	23.0	24.8	25.3	25.5
46.7	41.5	31.9	32.3	33.6	25.6	28.5	29.0
T	T	0.5	T	T	1.5	1.1	
0.9	1.6	3.7	2.9	2.3	2.7	2.8	4.7
11.6	17.4	25.1	20.0	24.6	25.1	26.3	25.3
T	0.5	T	T	1.9	2.8	1.5	1.9
T	0.8	0.8	T	2.9	5.2	3.0	1.8
					T	T	T
2.4	1.8	2.6	2.0	2.1	1.9	1.7	1.9
2.7	2.0	2.3	2.3	2.0	2.3	1.7	2.4
4.2	2.6	3.1	3.7	3.3	2.4	2.9	2.6
			T	T		2.0	
Q-9	Q-9	Q-9	Q-9	Q-9	Q-9	Q-9	Q-9

hydroxy acid; 16: 0, a straight-chain saturated acid of 16-carbon; 16: 1, a straight-chain unsatu-

0.5%.
chain; Q-9, nine units.

The 3-hydroxy fatty acids are found only in gram negative bacteria and not in gram positive bacteria, and the acids are mainly distributed in lipid A (*22*) or so-called bound lipids (*23*). There are two kinds of linkage of 3-hydroxy fatty acid to lipid A back bone (amide-bound and ester-bound 3-hydroxy fatty acids) (*22*). GALANOS et al. (*22*) suggested that the combination of these two kinds of 3-hydroxy fatty acids is specific to each bacterial species. YANO et al. (*23*) studied bound lipids of the species of Enterobacteriaceae and Vibrionaceae and of *Pseudomonas,*

Table 7. Grouping of *Pseudomonas* species based on 3-hydroxy fatty acid composition and quinone systems.

	3-Hydroxy fatty acids								Quinone systems
	3-OH 8:0	3-OH 10:0	3-OH 12:0	3-OH 14:0	3-OH 14:1	3-OH 16:0	3-OH *i*-11:0	3-OH *i*-13:0	
Group 1	−	+	+	−	−	−	−	−	Q-9
Group 2	−	−	−	+	−	+	−	−	Q-8
Group 3	−	+	−	−	−	−	−	−	Q-8
Group 4	−	−	+	+	−	−	−	−	Q-10
Group 5	−	−	+	−	−	−	+	+	Q-8
Group 6	−	−	−	−	−	−	−	−	Q-10
Group 7	−	−	−	+	−	−	−	−	Q-10
Group 8	+	−	−	−	−	−	−	−	Q-8
Group 9	−	+	+	+	+	−	−	−	Q-8

+, fatty acid detected; −, fatty acid not detected.
Abbreviations of fatty acids are the same as those described for Tables 5 and 6.

Table 8. Grouping of the strains of *Pseudomonas* species based on the 3-hydroxy fatty acid composition and quinone system.

Group 1
P. aeruginosa KS 0024, KS 0025, *P. alcaligenes* KS 0018, KS 0021, *P. aureofaciens* KS 0004, *P. azotoformans* KS 0034, *P. chlororaphis* KS 0015, *P. fluorescens* KS 0009, KS 0022, KS 0112, *P. fulva* KS 0029, KS 0030, "*P. lacunogenes*" KS 0036, KS 0037, KS 0222, *P. mendocina* KS 0097, *P. mucidolens* KS 0038, *P. nitroreducens* KS 0050, "*P. ochracea*" KS 0026, KS 0027, "*P. ovalis*" KS 0008, KS 0010, *P. putida* KS 0100, *P. straminea* KS 0028, KS 0270, *P. stutzeri* KS 0013, *P. syringae* pv. *coronafaciens* KS 0252, KS 0253, *P. syringae* pv. *eriobotryae* KS 0254, *P. syringae* pv. *japonica* KS 0263, KS 0264, *P. syringae* pv. *mori* KS 0259, KS 0260, *P. syringae* pv. *tabaci* KS 0265, *P. taetrolens* KS 0017, KS 0235, KS 0237, KS 0238, KS 0239, KS 0240.

Group 2
P. caryophylli KS 0250, *P. cepacia* KS 0052, KS 0233, KS 0234, *P. gladioli* pv. *gladioli* KS 0258, *P. solanacearum* KS 0261, KS 0262.

Group 3
P. acidovorans KS 0056, KS 0057, "*P. cruciviae*" KS 0005, "*P. dacunhae*" KS 0006, "*P. desmolytica*" KS 0054, *P. flava* KS 0231, *P. iners* KS 0046, KS 0047, *P. pseudoflava* KS 0232, *P. testosteroni* KS 0043, KS 0048, "*Comamonas terrigena*" KS 0020.

Group 4
P. diminuta KS 0016, KS 0242, KS 0243, *P. vesicularis* KS 0241.

Group 5
P. maltophilia KS 0001, KS 0002, KS 0131, *P. pictorum* KS 0271.

Group 6
P. paucimobilis KS 0300, KS 0301.

Group 7
"*P. extorquens*" KS 0111, "*P. rosea*" KS 0312, *Pseudomonas* sp. BP-22.

Group 8
P. palleronii KS 0230.

Group 9
P. avenae KS 0256, KS 0257.

Xanthomonas, Achromobacter, Alcaligenes, and *Acetobacter*, and showed the taxonomic significance of 3-hydroxy fatty acid composition of the bound lipids in these bacteria. In this paper we have focused on the 3-hydroxy fatty acid composition because of the biochemical importance of the acids. The taxonomic significance of the quinone system has been shown in various bacterial groups, such as coryneform bacteria (*24*), *Nocardia* species (*25*), *Bacillus* species (*26*), aerobic gram positive cocci (*27*, *28*), acetic acid bacteria (*16*), methanol-utilizing bacteria (*3*), and *Flavobacterium-Cytophaga* complex (*14*).

As a result of our series of studies (*1–3*, *5*, *6*), a good correlation has been found

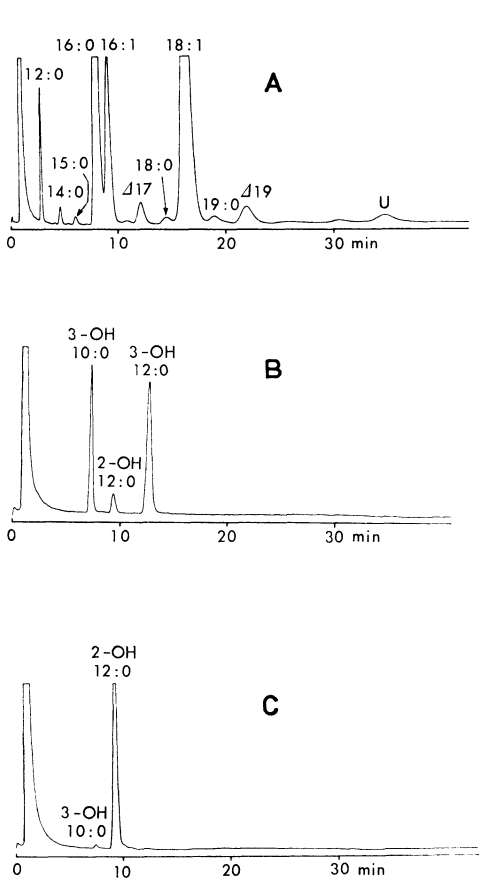


Fig. 1-1.

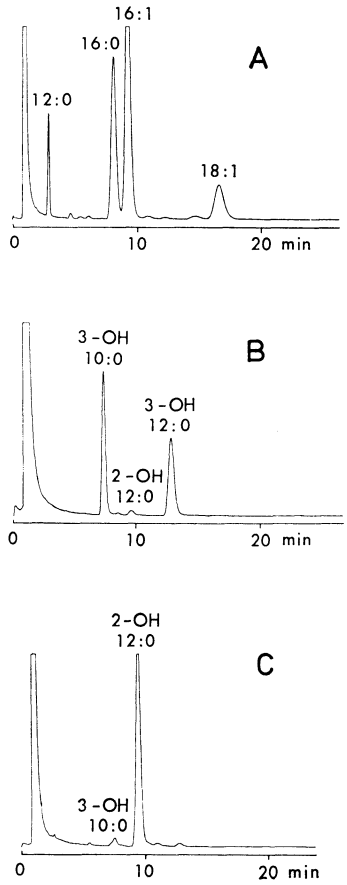


Fig. 1-2.

Fig. 1-1. Fatty acid profile of *Pseudomonas aeruginosa* KS 0025.
(A) non-polar acid fraction, (B) 3-hydroxy acid fraction, (C) 2-hydroxy fraction.
Fig. 1-2. Fatty acid profile of *Pseudomonas syringae* pv. *coronafaciens* KS 0252.
(A) non-polar acid fraction, (B) 3-hydroxy acid fraction, (C) 2-hydroxy acid fraction.

between the quinone system and the 3-hydroxy fatty acid composition in the species of the genus *Pseudomonas*. Ubiquinone-8 (Q-8) was found in the strains with 3-OH $C_{14:0}$ and 3-OH $C_{16:0}$; in the strains with 3-OH $C_{10:0}$; in the strains with 3-OH *i*-$C_{11:0}$, 3-OH $C_{12:0}$, and 3-OH *i*-$C_{13:0}$; in the strains with 3-OH $C_{8:0}$; and in the strains with 3-OH $C_{10:0}$, 3-OH $C_{12:0}$, 3-OH $C_{14:0}$, and 3-OH $C_{14:1}$. Ubiquinone-9 (Q-9) was found in the strains with 3-OH $C_{10:0}$ and 3-OH $C_{12:0}$. Ubiquinone-10 (Q-10) was found in the strains with 3-OH $C_{12:0}$ and 3-OH $C_{14:0}$; in the strains with no 3-hydroxy fatty acid; and in the strains with 3-OH $C_{14:0}$. Considering the 3-hydroxy fatty acid composition and quinone system, the 75 strains studied were further classified into nine separate groups, as shown in Table 7. The species and strain designations of each group are listed in Table 8.

Group 1. This group contains 40 strains (Table 8). They have 3-OH $C_{10:0}$ and 3-OH $C_{12:0}$, and Q-9. Fatty acid profiles of representative strains of this group are shown in Fig. 1-1 and 1-2. This group comprises all the fluorescent pigment-producing species of the genus *Pseudomonas* (*P. aeruginosa, Pseudomonas fluorescens, P. putida, Pseudomonas azotoformans* (*29*), *P. fulva* (*29*), *P. nitroreducens,* "*Pseudomonas ovalis*," *P. straminea* (*29*), and fluorescent pigment-producing phyto-

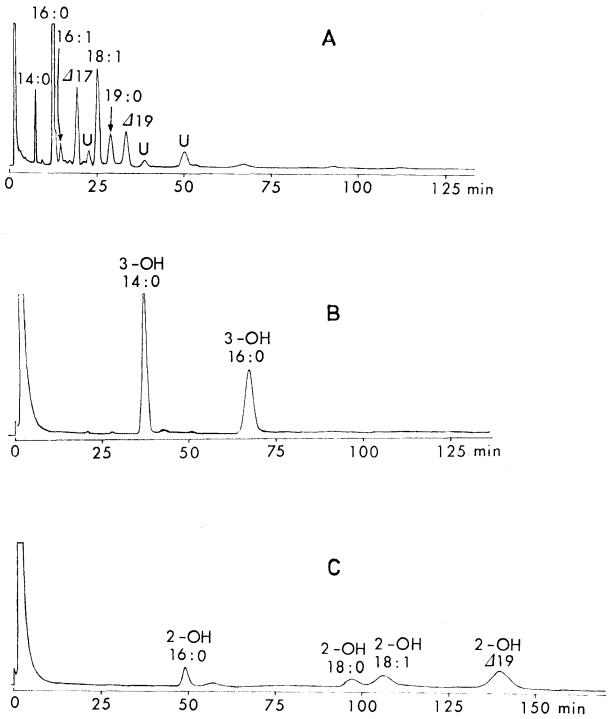


Fig. 2-1. Fatty acid profile of *Pseudomonas cepacia* KS 0052.
(A) non-polar acid fraction, (B) 3-hydroxy acid fraction, (C) 2-hydroxy acid fraction.

pathogenic species, *P. syringae* (*30*)). This group also comprises the fluorescent pigment-not-producing species *Pseudomonas alcaligenes*, "*P. lacunogenes*" (*31*), *P. mendocina* (*32*), "*P. ochracea*" (*31*), and *P. stutzeri* (*32*). Group 1 comprises several water-insoluble yellow pigment-producing species ("*P. lacunogenes*" and "*P. ochracea*" of the chromogenic group of the system of IIZUKA and KOMAGATA (*8*, *9*), and *P. fulva* (*29*), *P. straminea* (*29*), and *P. mendocina* (*32*)). The strains of this group are heterogeneous in utilization of carbon compounds (*10*, *30*, *32*).

Group 2. This group contains seven strains (Table 8). These strains have 3-OH $C_{14:0}$ and 3-OH $C_{16:0}$, and Q-8. Fatty acid profiles of representative strains are shown in Fig. 2-1 and 2-2. Non-fluorescent species, *P. caryophylli*, *P. cepacia*,

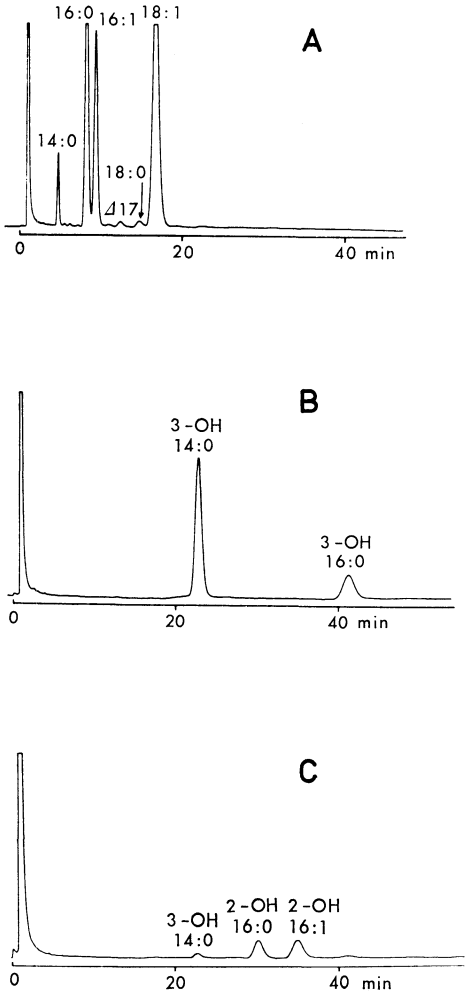


Fig. 2-2. Fatty acid profile of *Pseudomonas caryophylli* KS 0250.
(A) non-polar acid fraction, (B) 3-hydroxy acid fraction, (C) 2-hydroxy acid fraction.

P. gladioli, and *P. solanacearum* are included. This group comprises several phytopathogenic species (*P. caryophylli, P. gladioli,* and *P. solanacearum* (*33*, *34*)). The strains are heterogeneous in utilization of carbon compounds (*33*, *34*).

Group 3. This group contains 12 strains (Table 8). They have 3-OH $C_{10:0}$ and Q-8. Fatty acid profiles of representative strains are shown in Fig. 3-1 and 3-2. The species of the so-called acidovorans group (*10*), such as *P. acidovorans, P. testosteroni*, and "*Comamonas terrigena*," are included in this group. Group 3 also contains two water-insoluble yellow pigment-producing species, *P. flava* and *P. pseudoflava*. URAKAMI and KOMAGATA (*3*) grouped gram negative methanol-utilizing bacteria on the basis of phenotypic and chemotaxonomic characteristics. Polarly flagellated methanol-utilizing rods were divided into groups 1 and 2. As the strains of their group 1 have the same 3-hydroxy fatty acid composition (3-OH $C_{10:0}$) and quinone system (Q-8) as our group 3, their group 1 seems to be related to our group 3. However, these strains do not grow on nutrient broth and have special enzymes for utilizing methanol (*3*, *4*), and their G+C contents have a

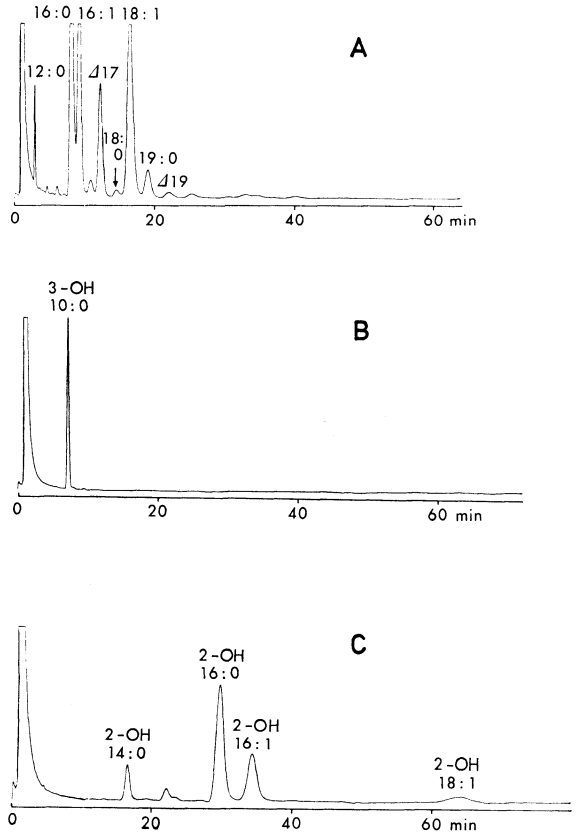


Fig. 3-1. Fatty acid profile of *Pseudomonas testosteroni* KS 0043.
(A) non-polar acid fraction, (B) 3-hydroxy acid fraction, (C) 2-hydroxy acid fraction.

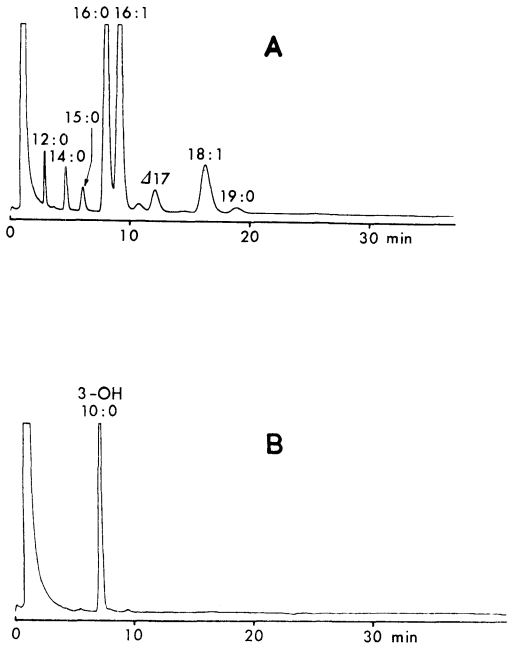


Fig. 3-2. Fatty acid profile of "*Comamonas terrigena*" KS 0020.
(A) non-polar acid fraction, (B) hydroxy acid fraction.

lower range (50–55%) (personal communication from T. Urakami, Niigata Research Laboratory, Mitsubishi Gas Chemical Co., Inc.) than that of *Pseudomonas* species (58–70% (*7*)). Therefore, cautious consideration should be required for including these strains in our group 3. The strains of this group are heterogeneous in utilization of carbon compounds (*10*, *35*).

Group 4. This group contains three strains of *P. diminuta* and one strain of *P. vesicularis* (Table 8). These strains have 3-OH $C_{12:0}$ and 3-OH $C_{14:0}$, and Q-10. The fatty acid profile of a representative strain is shown in Fig. 4. IKEMOTO et al. (*1*) showed that the cellular fatty acid composition of *P. diminuta* strains characteristically includes a small amount of *i*-$C_{15:0}$. The presence of *i*-$C_{15:0}$ in the strains of this group was confirmed in this study, and is considered to be a specific characteristic of this group. The strains of this group require growth factors and show heterogeneity in utilization of carbon compounds (*36*).

Group 5. This group contains three strains of *P. maltophilia* and *P. pictorum* KS 0271 (Table 8). All of the strains produce water-insoluble yellow pigment. They have 3-OH $C_{10:0}$, 3-OH *i*-$C_{11:0}$, 3-OH $C_{11:0}$, 3-OH *i*-$C_{12:0}$, 3-OH $C_{12:0}$, and 3-OH *i*-$C_{13:0}$. Quinone system is Q-8. The fatty acid profile of a representative strain is shown in Fig. 5. In the genus *Pseudomonas*, only the strains of this group show cellular fatty acid composition with a large amount of the branched-chain fatty acids *i*-$C_{15:0}$, *a*-$C_{15:0}$, *i*-$C_{16:0}$, and *i*-$C_{17:1}$.

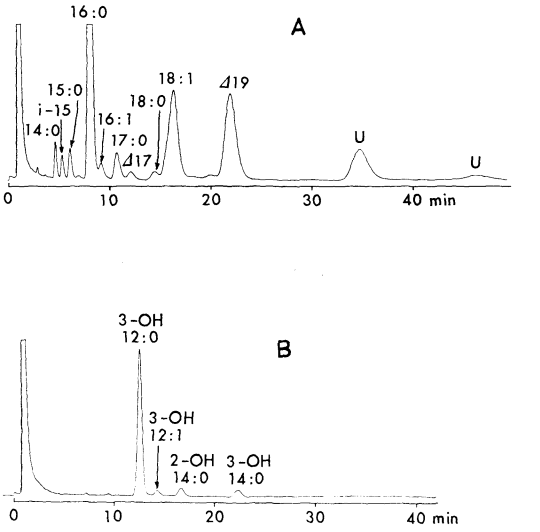


Fig. 4. Fatty acid profile of *Pseudomonas diminuta* KS 0016.
(A) non-polar acid fraction, (B) hydroxy acid fraction.

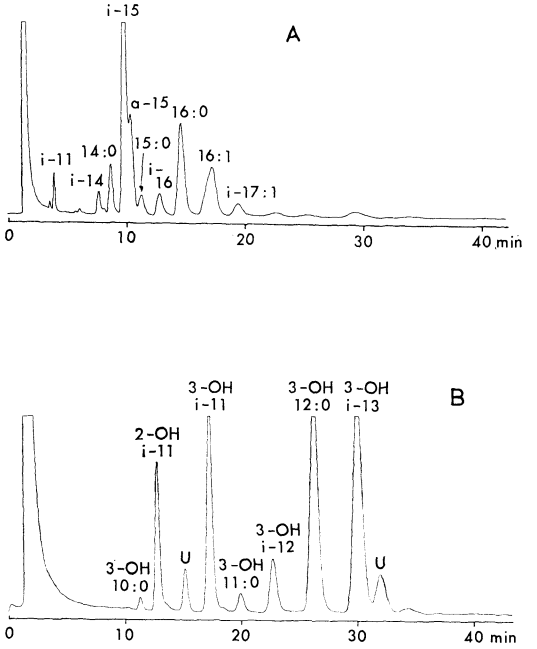


Fig. 5. Fatty acid profile of *Pseudomonas maltophilia* KS 0001.
(A) non-polar acid fraction, (B) hydroxy acid fraction.

Xanthomonas species appears to be closely related to this group with regard to the cellular fatty acid composition and quinone system, as pointed out by IKEMOTO et al. (*6*).

Group 6. This group contains *P. paucimobilis* KS 0300 and KS 0301. The strains produce yellow orange pigment. They have Q-10 and do not have 3-hydroxy fatty acid. The absence of 3-hydroxy fatty acid in the cells is an unusual property in gram negative bacteria. YABUUCHI et al. (*37*) and KAWAHARA et al. (*38*) also mentioned the absence of 3-hydroxy fatty acids in both bound lipids and extractable lipids of *P. paucimobilis*. KAWAHARA et al. (*38*) reported that the 2-hydroxy fatty acids may play the same roles as 3-hydroxy fatty acids on the lipid A of *P. paucimobilis*.

Group 7. This group includes the water-insoluble pink to red pigment-producing, methanol-utilizing "*P. extorquens*" KS 0111, "*P. rosea*" KS 0312, and *Pseudomonas* sp. BP-22. All the strains have 3-OH $C_{14:0}$ and Q-10. The fatty acid profile of "*P. extorquens*" KS 0111 is shown in Fig. 6. URAKAMI and KOMAGATA (*3*, *4*) included these strains in group 2 of their gram negative, methanol-utilizing bacteria groups. The three strains studied here are the representative strains of the three subgroups of the group 2 of URAKAMI and KOMAGATA (*4*). Therefore,

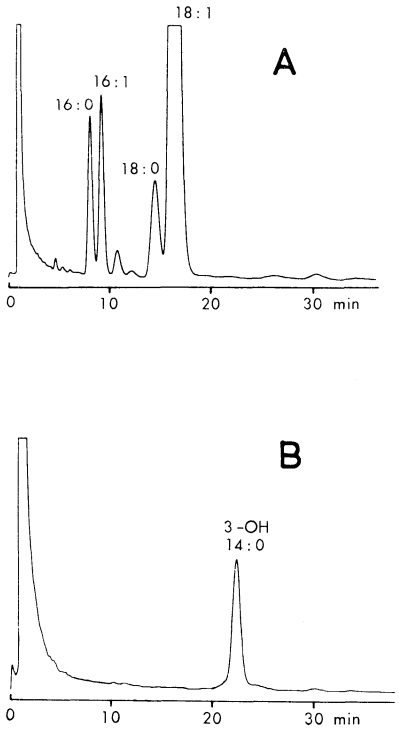
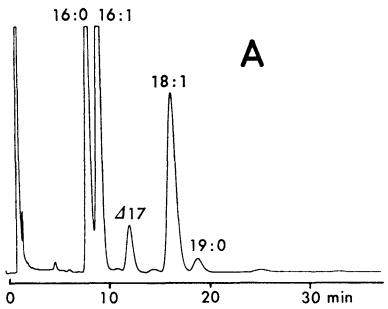


Fig. 6. Fatty acid profile of "*Pseudomonas extorquens*" KS 0111.
(A) non-polar acid fraction, (B) hydroxy acid fraction.

the other strains of their group 2 should also be included in our group 7.

Group 8. This group contains *P. palleronii* KS 0230, which has 3-OH $C_{8:0}$ and Q-8. The fatty acid profile of this strain is shown in Fig. 7. This group consists of only the one species, *P. palleronii*, and a few cultures of this species have been deposited in the culture collections. Therefore, isolation of many more strains of this group is needed to understand the interrelation between this group and other *Pseudomonas* species.

Group 9. This group contains *P. avenae* KS 0256 and KS 0257. They have 3-OH $C_{10:0}$, 3-OH $C_{12:0}$, 3-OH $C_{12:1}$, 3-OH $C_{14:0}$, and 3-OH $C_{14:1}$, and Q-8. The fatty acid profile of *P. avenae* KS 0256 is shown in Fig. 8. This group consists of only one species, *P. avenae*, and a few cultures of this species have been deposited in the culture collections. Therefore, isolation of many more strains is needed to understand the interrelation between this group and other *Pseudomonas* species.



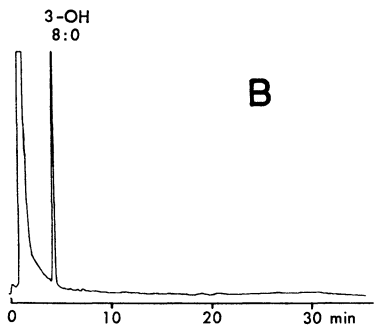
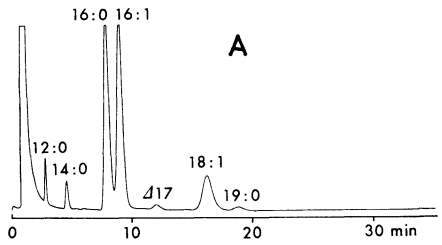


Fig. 7.



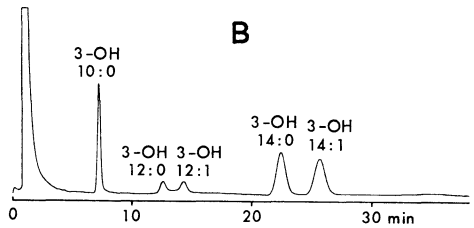


Fig. 8.

Fig. 7. Fatty acid profile of *Pseudomonas palleronii* KS 0230.
(A) non-polar acid fraction, (B) hydroxy acid fraction.

Fig. 8. Fatty acid profile of *Pseudomonas avenae* KS 0256.
(A) non-polar acid fraction, (B) hydroxy acid fraction.

Correlation between the present grouping based on 3-hydroxy fatty acid composition and quinone system and groups based on rRNA-DNA homology by PALLERONI et al. (39)

A good correlation is found between groups 1 through 5 of our grouping based on the 3-hydroxy fatty acid composition and quinone system and rRNA-DNA homology groups reported by PALLERONI et al. (*39*) (Table 9). PALLERONI et al. reported that the strains which showed no or little interrelationship in DNA-DNA homology showed relatively high indices in rRNA-DNA homology, and that five rRNA homology groups showed a little interrelation with one another in rRNA-DNA homology indices. YANO et al. (*40*) reported finding, as we did, that the five groups of PALLERONI et al. had peculiar lipid and fatty acid compositions. However, the taxonomic significance of the 3-hydroxy fatty acid composition was not stressed in their study. YAMADA et al. (*2*) showed, as confirmed in this study, that the quinone system of the *Pseudomonas* species coincides nicely with the grouping of PALLERONI et al. On the other hand, BYNG et al. (*41*) and WHITAKER et al. (*42, 43*) discriminated the five groups of PALLERONI et al. based on the comparative allostery of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase and enzymological patterning in tyrosine and phenylalanine biosynthesis, and they emphasized the adequacy of the grouping of PALLERONI et al. at a hierarchic level.

Table 9. Correlation between groups based on 3-hydroxy fatty acid composition and quinone systems studied by OYAIZU and KOMAGATA and the rRNA-DNA homology groups reported by PALLERONI *et al.*

Groups based on 3-hydroxy fatty acid composition and quinone systems	rRNA-DNA homology groups of PALLERONI *et al.*				
	Group I	Group II	Group III	Group IV	Group V
Group 1	*P. aeruginosa* *P. alcaligenes* *P. fluorescens* *P. mendocina* *P. putida* *P. stutzeri*				
Group 2		*P. caryophylli* *P. cepacia* *P. gladioli* (synonym of "*P. marginata*") *P solanacearum*			
Group 3			*P. acidovorans* *P. testosteroni*		
Group 4				*P. diminuta* *P. vesicularis*	
Group 5					*P. maltophilia*

Specific names are limited to those appeared in the study of PALLERONI et al. (*39*).

However, a difference is found between the results shown in this study and the results of BYNG et al. and WHITAKER et al., in that in their study *P. palleronii* was included in the group consisting of *P. acidovorans* and *P. testosteroni*, namely, PALLERONI's group III. In our study, the type strain of *P. palleronii* (KS 0230= ATCC 17724) had a 3-hydroxy fatty acid composition (3-OH $C_{8:0}$) different from *P. acidovorans* and *P. testosteroni* (3-OH $C_{10:0}$).

The species of groups 6 through 9 described in this study are not included in the study of PALLERONI et al. Group 6, *Pseudomonas paucimobilis*, shows little relationship to PALLERONI's five groups in having no 3-hydroxy fatty acid. Group 7, red to pink pigment-producing methanol-utilizers, has bacteriochlorophyll a (*44*), and shows cellular fatty acid composition distinctly different from the strains of any of PALLERONI's groups (*3*, *5*). Since few taxonomic studies have been done on *P. palleronii* (group 8) and *P. avenae* (group 9), discussion of the relation of the two species to any of the PALLERONI's five groups is rather limited. Therefore, more extensive study including rRNA-DNA homology on *P. palleronii* and *P. avenae* will be required.

In conclusion, the genus *Pseudomonas* is considered to be a heterogeneous taxon, and division at the genus level is expected. The grouping described in this paper may be one way to solve this problem.

Relation of the genus Pseudomonas to related genera

Previously OYAIZU and KOMAGATA (*14*) reported the quinone system and the cellular fatty acid composition of the strains of species in the *Flavobacterium-Cytophaga* complex. The strains with high G+C content showed close relationship to the genera *Pseudomonas* and *Alcaligenes*. The strains resembling *Pseudomonas* species (cluster 1 and 3 of the *Flavobacterium-Cytophaga* complex (*14*)) did not show motility in the hanging drop. The strains with peritrichous flagella (cluster 2 (*14*)) were identified as *Alcaligenes* species because of the flagellation and chemotaxonomic characteristics. The strains of cluster 2 were divided into two phenovars, phenovar 2-1 and phenovar 2-2, on the basis of utilization of carbon compounds and hydroxy fatty acid composition. The strains in phenovar 2-1 have the same 3-hydroxy fatty acids (3-OH $C_{14:0}$ and 3-OH $C_{16:0}$) and the same quinone system (Q-8) as those of group 2 in the genus *Pseudomonas* described in this study. The taxonomic significance of 3-hydroxy fatty acid composition and quinone system is confirmed in the genus *Pseudomonas*. Therefore, taxonomic reevaluation of motility and flagellation is needed for the better classification of the genera *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, and related bacteria. WHITAKER et al. (*43*) mentioned that *Alcaligenes eutrophus* resembles the species of group II of PALLERONI et al., and that *Alcaligenes paradoxus* and *Alcaligenes faecalis* resemble the species of group III of PALLERONI et al. in the point of enzymological patterning in biosynthesis of tyrosine and phenylalanine. STACKEBRANDT and WOESE (*45*) reviewed the phylogenetic relation of the purple photosynthetic bacteria and related bacteria

including *Pseudomonas* species based on the similarities of 16S rRNA catalogues. They mentioned that *Pseudomonas* species were divided into five clusters, and that each of these five cluster is closely related to other genera, such as *Rhodopseudomonas*, *Rhodospirillum*, *Alcaligenes*, *Lysobacter*, and *Azotobacter*.

Considering the heterogeneity and the phylogenetic relations to other genera of the genus *Pseudomonas*, more extensive study including a wide range of bacterial taxa will be required for better understanding of the genus.

The authors wish to thank K. Nishiyama and K. Takahashi, National Institute of Agricultural Sciences, Tsukuba, Ibaraki 305, Japan, for supplying the cultures.

REFERENCES

1) S. Ikemoto, H. Kuraishi, K. Komagata, R. Azuma, T. Suto, and H. Murooka, *J. Gen. Appl. Microbiol.*, **24**, 199 (1978).
2) Y. Yamada, H. Takinami-Nakamura, Y. Tahara, H. Oyaizu, and K. Komagata, *J. Gen. Appl. Microbiol.*, **28**, 7 (1982).
3) T. Urakami and K. Komagata, *J. Gen. Appl. Microbiol.*, **25**, 343 (1979).
4) T. Urakami and K. Komagata, *J. Gen. Appl. Microbiol.*, **27**, 381 (1981).
5) S. Ikemoto, K. Katoh, and K. Komagata, *J. Gen. Appl. Microbiol.*, **24**, 41 (1978).
6) S. Ikemoto, K. Suzuki, T. Kaneko, and K. Komagata, *Int. J. Syst. Bacteriol.*, **30**, 437 (1980).
7) M. Doudoroff and N. J. Palleroni, *In* Bergey's Manual of Determinative Bacteriology, 8th ed., ed. by R. E. Buchanan and N. E. Gibbons, The Williams and Wilkins Co., Baltimore (1974), p. 217.
8) H. Iizuka and K. Komagata, *J. Gen. Appl. Microbiol.*, **9**, 73 (1963).
9) H. Iizuka and K. Komagata, *J. Gen. Appl. Microbiol.*, **9**, 83 (1963).
10) R. Y. Stanier, N. J. Palleroni, and M. Doudoroff, *J. Gen. Microbiol.*, **43**, 159 (1966).
11) V. B. D. Skerman, V. McGowan, and P. H. A. Sneath (ed.), *Int. J. Syst. Bacteriol.*, **30**, 225 (1980).
12) R. Hugh, *Int. J. Syst. Bacteriol.*, **31**, 195 (1981).
13) N. J. Palleroni and B. Holmes, *Int. J. Syst. Bacteriol.*, **31**, 479 (1981).
14) H. Oyaizu and K. Komagata, *J. Gen. Appl. Microbiol.*, **27**, 57 (1981).
15) K. Suzuki, K. Saito, A. Kawaguchi, S. Okuda, and K. Komagata, *J. Gen. Appl. Microbiol.*, **27**, 261 (1981).
16) Y. Yamada, K. Aida, and T. Uemura, *J. Gen. Appl. Microbiol.*, **15**, 181 (1969).
17) G. Wilkinson, L. Galbraith, and G. A. Lightfoot, *Eur. J. Biochem.*, **33**, 158 (1973).
18) C. M. Kaltenbach, C. W. Moss, and R. E. Weaver, *J. Clin. Microbiol.*, **1**, 339 (1975).
19) C. W. Moss and S. B. Dees, *J. Clin. Microbiol.*, **4**, 492 (1976).
20) C. W. Moss, S. B. Samuels, J. Liddle, and R. M. McKinney, *J. Bacteriol.*, **114**, 1018 (1973).
21) C. W. Moss and S. B. Dees, *J. Chromatogr.*, **112**, 595 (1975).
22) C. Galanos, O. Lüderitz, E. T. Rietshel, and O. Westphal, *Int. Rev. Biochem.*, **14**, 239 (1977).
23) T. Yano, M. Ohno, M. Masui, and E. Yabuuchi, *Shishitsu Seikagaku Kenkyu*, **17**, 213 (1975).
24) Y. Yamada, G. Inouye, Y. Tahara, and K. Kondo, *J. Gen. Appl. Microbiol.*, **22**, 203 (1976).
25) Y. Yamada, T. Ishikawa, Y. Tahara, and K. Kondo, *J. Gen. Appl. Microbiol.*, **23**, 207 (1977).

26) M. WATANUKI and K. AIDA, *J. Gen. Appl. Microbiol.*, **18**, 469 (1972).
27) Y. YAMADA, G. INOUYE, Y. TAHARA, and K. KONDO, *J. Gen. Appl. Microbiol.*, **22**, 227 (1976).
28) Y. YAMADA, H. TAKINAMI, Y. TAHARA, and K. KONDO, *J. Gen. Appl. Microbiol.*, **23**, 331 (1977).
29) H. IIZUKA and K. KOMAGATA, *Nippon Nogei Kagaku Kaishi*, **37**, 137 (1963).
30) I. MISAGHI and R. G. GROGAN, *Phytopathology*, **59**, 1436 (1969).
31) H. IIZUKA and K. KOMAGATA, *Nippon Nogei Kagaku Kaishi*, **37**, 71 (1963).
32) N. J. PALLERONI, M. DOUDOROFF, R. Y. STANIER, R. E. SOLANES, and M. MANDEL, *J. Gen. Microbiol.*, **60**, 215 (1970).
33) R. W. BALLARD, N. J. PALLERONI, M. DOUDOROFF, R. Y. STANIER, and M. MANDEL, *J. Gen. Microbiol.*, **60**, 199 (1970).
34) N. J. PALLERONI and M. DOUDOROFF, *J. Bacteriol.*, **107**, 690 (1971).
35) G. AULING, M. REH, C. M. LEE, and H. G. SCHLEGEL, *Int. J. Syst. Bacteriol.*, **28**, 82 (1978).
36) R. W. BALLARD, M. DOUDOROFF, R. Y. STANIER, and M. MANDEL, *J. Gen. Microbiol.*, **53**, 349 (1968).
37) E. YABUUCHI, E. TANIMURA, A. OHYAMA, I. YANO, and A. YAMAMOTO, *J. Gen. Appl. Microbiol.*, **25**, 95 (1979).
38) K. KAWAHARA, K. UCHIDA, and K. AIDA, *Biochim. Biophys. Acta*, **712**, 571 (1982).
39) N. J. PALLERONI, R. KUNISAWA, R. CONTOPOULOU, and M. DOUDOROFF, *Int. J. Syst. Bacteriol.*, **23**, 333 (1973).
40) I. YANO, M. SHIMOKAWA, Y. OHNO, M. MASUI, Y. YABUUCHI, and K. KATO, *Shishitsu Seikagaku Kenkyu*, **18**, 51 (1976).
41) G. S. BYNG, R. J. WHITAKER, R. L. GHERNA, and R. A. JENSEN, *J. Bacteriol.*, **144**, 247 (1980).
42) R. J. WHITAKER, G. S. BYNG, R. L. GHERNA, and R. A. JENSEN, *J. Bacteriol.*, **145**, 752 (1981).
43) R. J. WHITAKER, G. S. BYNG, R. L. GHERNA, and R. A. JENSEN, *J. Bacteriol.*, **147**, 526 (1981).
44) K. SATO and S. SHIMIZU, *Agric. Biol. Chem.*, **43**, 1669 (1979).
45) E. STACKEBRANDT and C. R. WOESE, *In* Molecular and Cellular Aspects of Microbial Evolution, ed. by M. J. CARLILE, J. F. COLLINS, and B. E. B. MOSELY, Cambridge University Press, Cambridge (1981), p. 1.
46) K. NISHIYAMA, K. OHATA, Y. TSUCHIYA, and A. OHUCHI, List of cultures in the plant pathology division of National Institute of Agricultural Sciences, Misc. Publ. Natl. Inst. Agric. Sci., Ser. C No. 3, National Institute of Agricultural Sciences, Tsukuba, Japan (1981).