Numerical Taxonomy of *Pseudomonas alcaligenes*, *P. pseudoalcaligenes*, *P. mendocina*, *P. stutzeri*, and Related Bacteria

FRANCOISE GAVINI,^{1*} BARRY HOLMES,³ DANIEL IZARD,^{1,4} AMOR BEJI,¹ ANNIE BERNIGAUD,¹ AND EDMOND JAKUBCZAK²

Institut National de la Santé et de la Recherche Médicale Unité 146,¹ and Ecole Nationale Supérieure de l'Industrie Alimentaire,² Domaine du Centre d'Enseignement et de Recherches Techniques en Industrie Alimentaire, F-59651 Villeneuve d'Ascq Cedex, France; National Collection of Type Cultures, Central Public Health Laboratory, London NW9 5HT, United Kingdom³; and Service de Bactériologie A, Faculté de Médecine, F-59045 Lille Cédex, France⁴

A numerical phenotypic analysis, in which the unweighted pair group average linkage method and Dice similarity coefficient were used, was performed on 155 strains received as *Pseudomonas alcaligenes*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas mendocina*, or *Pseudomonas stutzeri*. These organisms are the clinically important nonfluorescent species belonging to ribosomal ribonucleic acid group I of Palleroni and co-workers. Six major clusters, which could be further divided into 20 subclusters, were formed. Most strains received as *P. alcaligenes* fell into three subclusters (subclusters A1, A2, and B1), whereas strains received as *P. pseudoalcaligenes* were mainly classified in two other subclusters (subclusters C2 and C3). All but two strains (subcluster D1) of organisms received as *P. mendocina* were grouped in subcluster D2. Most of the 45 strains received as *P. stutzeri* were contained in a large subcluster, subcluster E2 (39 strains). Strains belonging to fluorescent pseudomonad species (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Pseudomonas putida*), which were included in the analysis for control purposes, were contained in one cluster, which comprised seven subclusters.

During recent years, *Pseudomonas* spp. strains have been studied with increasing interest because of their importance in medical and food microbiology and in phytopathology. Since the classical study of Stanier et al. (30) on *Pseudomonas* taxonomy, most of the analyses performed on these strains have pointed to the genomic heterogeneity of the genus (5, 17, 35). However, very few studies have been carried out at the species level to revise the phenotypic definitions of these organisms and to determine their genomic positions by deoxyribonucleic acid (DNA)-DNA hybridization. Only fluorescent *Pseudomonas* spp. strains and strains isolated from meat have been the subjects of several taxonomic studies at the species level (2, 3, 14–16, 24, 26, 34). Although the nutritional characteristics given by Stanier et al. (30) are still potentially the most useful for differentiation, the lack of other phenotypic data leads to difficulties in developing identification schemes.

The aim of this study was to define, by using a numerical phenotypic analysis, species known to belong to ribosomal ribonucleic acid group I (18) on the basis of DNA-ribosomal ribonucleic acid hybridization (17), particularly the clinically important species of this group, which were received as *Pseudomonas alcaligenes*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas mendocina*, or *Pseudomonas stutzeri*. Later, we intend to study the phenotypic groups by using DNA-DNA hybridization.

MATERIALS AND METHODS

Bacterial strains. A total of 155 strains were included in the analysis. These organisms comprised type and other reference strains received from diverse culture collections as *P. alcaligenes*, *P. pseudoalcaligenes*, *P. mendocina*, or *P. stutzeri*. Strains belonging to *Pseudomonas fluorescens* biovars A, B, C, F, and G, *Pseudomonas chlororaphis*, *Pseu-*

domonas aureofaciens, *Pseudomonas putida* biovars A and B, and *Pseudomonas aeruginosa* were included for control purposes.

Details concerning the strains, including their reference numbers and clinical sources (where known), are given in Table 1.

Phenotypic characterization. In all, 215 characters were determined. Seven of these, which were either positive or negative for all strains, were not included in the numerical analysis. Of the 208 characters coded, some were subdivided, such as acidification or alkalization of media containing carbohydrates, which corresponded to two unit characters; others were grouped together, such as diffusible pigment on King medium A or King medium B or both, and coded so as to give a single quantitative multistate character (13). The following tests were performed as described previously (7, 8): motility, presence of oxidase, growth at 4°C and in the presence of different concentrations of sodium chloride (0, 0.8, 3, 5, and 7%, wt/vol), indole and acetoin production, utilization of citrate (Simmons method), nitrate and nitrite reduction, production of urease and phenylalanine deaminase, mucate and malonate utilization, esculin and starch hydrolysis, hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside and *o*-nitrophenyl-β-D-xylopyranoside, deoxyribonuclease, and hydrolysis of Tween 80.

The other characters were determined as described below. Growth was determined at 10 and 42°C (on nutrient agar; the results were recorded for up to 15 and 8 days, respectively), on cetrimide agar (trimethylammonium bromide; Merck, Nogent-sur-Marne, France), and on nutrient agar containing 1% (wt/vol) 2,3,5-triphenyltetrazolium chloride; the results for the two latter tests were read within 2 to 8 days. The presence of diffusible pigment was tested on King media A and B. Production of lipase and production of precipitation around colonies on egg yolk agar were demonstrated on tributyrin medium (tributyrin agar; Merck) and on nutrient agar containing Bacto egg yolk enrichment 50% (Difco

* Corresponding author.

TABLE 1. Strains used in this study

Cluster	Subcluster	Culture collection or other reference no. ^a	Name as received	Isolated from:
A (12 strains)	A1 (7 strains)	CCUG 12941	<i>P. alcaligenes</i>	Bronchial aspirate
		G 4367	<i>P. alcaligenes</i>	?
		G 3530	<i>P. alcaligenes</i>	Blood
		G 3603	<i>P. alcaligenes</i>	Environment
		G 3621	<i>P. alcaligenes</i>	Environment
		G 4804	<i>P. alcaligenes</i>	?
		G 4519	<i>P. alcaligenes</i>	Blood
	A2 (3 strains)	G 4713	<i>P. pseudoalcaligenes</i>	?
		G 4522	<i>P. pseudoalcaligenes</i>	?
		G 4767	<i>P. pseudoalcaligenes</i>	?
	Unclustered	CIP 55-111 (= Stanier 297)	<i>P. pseudoalcaligenes</i>	Blood (from rabbit)
		G 4570	<i>P. pseudoalcaligenes</i>	?
B (12 strains)	B1 (10 strains)	CCUG 6697B	<i>P. pseudoalcaligenes</i>	Drain
		CCUG 13700B	<i>P. mendocina</i>	Ear
		API 012-02-84	<i>P. alcaligenes</i>	?
		CCUG 5004	<i>P. pseudoalcaligenes</i>	Water
		CCUG 1316	<i>P. alcaligenes</i>	?
		CCUG 15505	<i>P. alcaligenes</i>	?
		CCUG 1315	<i>P. alcaligenes</i>	?
		ATCC 14909 ^T (= NCTC 10367 ^T = CCEB 795 ^T = Stanier 142 ^T)	<i>P. alcaligenes</i>	Water
		API 111-06-82	<i>P. alcaligenes</i>	?
		G 3512	<i>P. alcaligenes</i>	Environment
	Unclustered	CCUG 7819	<i>P. pseudoalcaligenes</i>	Water
		R 26-76	<i>P. pseudoalcaligenes</i>	?
C (48 strains)	C1 (2 strains)	R 3-82	<i>P. pseudoalcaligenes</i>	Bronchial wash
		CCUG 15506	<i>P. alcaligenes</i>	?
	C2 (14 strains)	P 490	<i>P. pseudoalcaligenes</i>	?
		G 5035	<i>P. pseudoalcaligenes</i>	?
		API 010-07-84	<i>P. pseudoalcaligenes</i>	?
		API 243-03-84	<i>P. pseudoalcaligenes</i>	?
		API 241-03-84	<i>P. pseudoalcaligenes</i>	?
		CCUG 299	<i>P. pseudoalcaligenes</i>	Water
		CCUG 1103	<i>P. pseudoalcaligenes</i>	?
		API 012-07-84	<i>P. pseudoalcaligenes</i>	?
		API 014-07-84	<i>P. pseudoalcaligenes</i>	?
		API 103-04-76	<i>P. pseudoalcaligenes</i>	?
		API 013-07-84	<i>P. pseudoalcaligenes</i>	?
		API 235-04-76	<i>P. pseudoalcaligenes</i>	?
		P 1363	<i>P. pseudoalcaligenes</i>	?
		API 242-03-84	<i>P. pseudoalcaligenes</i>	?
	C3 (25 strains)	CCUG 15 238	<i>P. pseudoalcaligenes</i>	?
		API 231-04-76	<i>P. pseudoalcaligenes</i>	?
		CCUG 12938 (= G 4252)	<i>P. pseudoalcaligenes</i>	Bronchial wash
		API 009-07-84	<i>P. alcaligenes</i>	?
		API 008-07-84	<i>P. alcaligenes</i>	?
		R 4-83	<i>P. pseudoalcaligenes</i>	?
		P 1757	<i>P. pseudoalcaligenes</i>	?
		API 240.03.84	<i>P. pseudoalcaligenes</i>	?
		P 1975	<i>P. pseudoalcaligenes</i>	?
		DSM 50189 (= CIP 61-21 = Stanier 65)	<i>P. pseudoalcaligenes</i>	Contaminant of growth medium
		API 011-07-84	<i>P. pseudoalcaligenes</i>	?
		API 028-12-77	<i>P. pseudoalcaligenes</i>	?
		CCUG 15284	<i>P. pseudoalcaligenes</i>	?
		CCUG 13432	<i>P. pseudoalcaligenes</i>	Industrial cooling fluid
		API 141-05-82	<i>P. pseudoalcaligenes</i>	?
		CIP 66-15 (= Stanier 299)	<i>P. pseudoalcaligenes</i>	?
		CCUG 6916	<i>P. pseudoalcaligenes</i>	Industrial cooling fluid
		P 1249	<i>P. pseudoalcaligenes</i>	?
		R 4-82	<i>P. pseudoalcaligenes</i>	Gastric sample
		G 4926	<i>P. pseudoalcaligenes</i>	?
		CIP 66-14 ^T (= ATCC 17440 ^T = NCIB 9946 ^T = DSM 50188 ^T = CCUG 726 ^T = Stanier 63 ^T)	<i>P. pseudoalcaligenes</i>	Sinus drainage
		API 014-05-82	<i>P. pseudoalcaligenes</i>	?

Continued on following page

TABLE 1—Continued

Cluster	Subcluster	Culture collection or other reference no. ^a	Name as received	Isolated from:
D (13 strains)	C4(2 strains)	CIP 60-76 (= ATCC 17443 = Stanier 66)	<i>P. pseudoalcaligenes</i>	Pharyngeal sample
		ATCC 12815 (= CCUG 1840 = Stanier 417)	<i>P. pseudoalcaligenes</i>	Buccal cavity
		CCUG 793	<i>P. pseudoalcaligenes</i>	?
		G 4739	<i>P. pseudoalcaligenes</i>	?
	C5(2 strains)	CCUG 2087 (= ATCC 8062)	<i>P. oleovorans</i>	?
		CCUG 15237	<i>P. pseudoalcaligenes</i>	?
	Unclustered	CCUG 5181	<i>P. pseudoalcaligenes</i>	Industrial cooling fluid
		CCUG 15481	<i>P. alcaligenes</i>	?
		R 74-80	<i>P. pseudoalcaligenes</i>	Medical origin
	D1(2 strains)	R 76-80	<i>P. pseudoalcaligenes</i>	Blood culture
		MB 78708	<i>P. stutzeri</i>	Clinical isolate
	D2(10strains)	R 5-84	Denitrifying <i>Pseudomonas</i> sp. strain	Sputum
		G 2084	<i>P. mendocina</i>	?
		G 809	<i>P. mendocina</i>	?
E (45 strains)	Unclustered	ATCC 25413	<i>P. mendocina</i>	Water enrichment with sebacate as carbon source
		CCUG 2028 (= G 847)	<i>P. mendocina</i>	Urine
		R 3-84	<i>P. mendocina</i>	Leg ulcer
	E1(3 strains)	CIP 75-20 (= CCUG 12439)	<i>P. mendocina</i>	Soil enrichment with L-tartrate as carbon source
		CIP 75-22 (= CCUG 12441)	<i>P. mendocina</i>	Water enrichment with L-tartrate as carbon source
		ATCC 25412 (= CIP 75-19 = CCUG 5916)	<i>P. mendocina</i>	Water enrichment with sebacate as carbon source
		NCTC 10897	<i>P. mendocina</i>	?
		ATCC 25411 ^T (= CIP 75-21 ^T = CCEB 849 ^T = CCUG 1781 ^T)	<i>P. mendocina</i>	Soil enrichment with ethanol as carbon source
		CCUG 11527	<i>P. pseudoalcaligenes</i>	Sewage plant
		CIP 67-16	<i>P. stutzeri</i>	Dog; metritis
		CIP 67-14	<i>P. stutzeri</i>	Blood culture
		CIP 67-17	<i>P. stutzeri</i>	Blood culture
		CIP 67-2	<i>P. stutzeri</i>	Sputum
		M B63240	<i>P. stutzeri</i>	Clinical isolate
		CIP 67-10	<i>P. stutzeri</i>	Nasal swab
		G 4582	<i>P. stutzeri</i>	Clinical isolate
		M B74557	<i>P. stutzeri</i>	Clinical isolate
		M B70266	<i>P. stutzeri</i>	Clinical isolate
		M B68575	<i>P. stutzeri</i>	Clinical isolate
		M B24417	<i>P. stutzeri</i>	Clinical isolate
	E2(39strains)	M B53321	<i>P. stutzeri</i>	Clinical isolate
		M B10789	<i>P. stutzeri</i>	Clinical isolate
		M B81655	<i>P. stutzeri</i>	Clinical isolate
		M B53322	<i>P. stutzeri</i>	Clinical isolate
		G 4505	<i>P. stutzeri</i>	?
		G 4865	<i>P. stutzeri</i>	?
		G 4555	<i>P. stutzeri</i>	?
		G 4569	<i>P. stutzeri</i>	?
		G 4952	<i>P. stutzeri</i>	?
		G 4902	<i>P. stutzeri</i>	?
		G 4949	<i>P. stutzeri</i>	?
		M B68122	<i>P. stutzeri</i>	Clinical isolate
		M B37129	<i>P. stutzeri</i>	Clinical isolate
		M B60051	<i>P. stutzeri</i>	Clinical isolate
		M 58282	<i>P. stutzeri</i>	Clinical isolate
		M B39976	<i>P. stutzeri</i>	Clinical isolate
		M B41133	<i>P. stutzeri</i>	Clinical isolate
		M B67358	<i>P. stutzeri</i>	Clinical isolate
		G 4760	<i>P. stutzeri</i>	?
		M B78048	<i>P. stutzeri</i>	?

Continued on following page

TABLE 1—Continued

Cluster	Subcluster	Culture collection or other reference no. ^a	Name as received	Isolated from:
F (23 strains)		R 9-83	<i>P. stutzeri</i>	?
		CCEB 795	<i>P. alcaligenes</i>	Nematode
		G 4641	<i>P. stutzeri</i>	?
		CCUG 2288	<i>P. stutzeri</i>	Ear
		ATCC 17588 ^T (= CCEB 859 ^T = Stanier 221 ^T)	<i>P. stutzeri</i>	Spinal fluid
		CCUG 227 (= NCTC 10450)	<i>P. stutzeri</i>	Leg ulcer
		CIP 67-15	<i>P. stutzeri</i>	?
		CCEB 716	<i>P. stutzeri</i>	?
		CCEB 522 (= CIP 63-21)	<i>P. stutzeri</i>	?
		NCTC 10475	<i>P. stutzeri</i>	?
		G 4650	<i>P. stutzeri</i>	?
	E3 (2 strains)	G 4977	<i>P. stutzeri</i>	?
		G 4518	<i>P. stutzeri</i>	?
	Unclassified	M T34265	<i>P. stutzeri</i>	Clinical isolate
	F1 (2 strains)	CCUG 1317	<i>P. putida</i> biovar B	Soil
		ATCC 17430	<i>P. putida</i> biovar B	?
	F2 (2 strains)	DSM 50208 (= ATCC 17485)	<i>P. putida</i> biovar A	?
		CIP 52-191 ^T (= ATCC 12633 ^T)	<i>P. putida</i> biovar A	Soil enrichment with lactate as sole carbon source
	F3 (4 strains)	M B66263	<i>P. stutzeri</i>	Clinical isolate
		M B40690	<i>P. stutzeri</i>	Clinical isolate
		M S13419	<i>P. mendocina</i>	Clinical isolate
		M K5	<i>P. mendocina</i>	?
	F4 (2 strains)	CIP 76.110 (= ATCC 27853)	<i>P. aeruginosa</i>	Blood culture
		CIP 63-52 ^T (= ATCC 10145 ^T = CCEB 481 ^T)	<i>P. aeruginosa</i>	?
Unclustered	F5 (2 strains)	ATCC 17397 (= ATCC 25323 = DSM 50091 = CIP 73-25)	<i>P. fluorescens</i> biovar A	Tap water
		ATCC 13525 ^T (= CIP69-13 ^T)	<i>P. fluorescens</i> biovar A	Pre-filter tanks
	F6 (2 strains)	CCEB 518 ^T (= ATCC 13985) ^T	<i>P. aureofaciens</i>	?
		DSM 50083 ^T DSM 50083 ^T (= CCEB 554 ^T = ATCC 9446 ^T = CIP 63-22 ^T)	<i>P. chlororaphis</i>	Plate contaminant
	F7 (2 strains)	ATCC 17571	<i>P. fluorescens</i> biovar C	Polluted seawater
		ATCC 17559 (= CCUG 1319 = Stanier 91)	<i>P. fluorescens</i> biovar C	?
	Unclassified	M K7	<i>P. mendocina</i>	?
		ATCC 17386	<i>P. fluorescens</i> biovar G	Tryptophan-enriched water
		ATCC 17826 (= DSM 50106)	<i>P. fluorescens</i> biovar B	Seawater
		DSM 50148 (= ATCC 17533)	<i>P. fluorescens</i> biovar G	Soil
		DSM 50145 (= ATCC 12983)	<i>P. fluorescens</i> biovar F	?
		CUETM 85-99	<i>Pseudomonas</i> sp.	Water
		ATCC 17815	<i>P. fluorescens</i> biovar B	?
		CCEB 713	<i>P. alcaligenes</i>	?
		ATCC 33513	<i>P. alcaligenes</i>	Nitrified poultry manure

^a API, API System, La Balme les Grottes, Montalieu Vercieu, France; ATCC, American Type Culture Collection, Rockville, Md; CCEB, Culture Collection of Entomogenous Bacteria, Prague, Czechoslovakia; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; CIP, Collection of the Institut Pasteur, Paris, France; CUETM, Collection Unité Ecotoxicologie Microbienne, Villeneuve d'Ascq, France; DSM, Deutscher Sammlung von Mikroorganismen, Gottingen, Federal Republic of Germany; G, G. L. Gilardi, Hospital for Joint Diseases and Medical Center, New York, N.Y.; M, H. Monteil, Université Louis Pasteur, Strasbourg, France; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; NCTC, National Collection of Type Cultures, London, England; P, M. J. Pickett, University of California, Los Angeles; R, C. Richard, Institut Pasteur, Paris, France; Stanier, R.Y. Stanier (see reference 30).

Laboratories, Detroit, Mich.), respectively; both tests were read for up to 5 days. Hydrolysis of tyrosine and hydrolysis of gelatin were determined on plates containing nutrient agar supplemented with 5% (wt/vol) tyrosine and by the plate method of Stolp and Gadkari (31), respectively. Tetrathionate reduction, anaerobic respiration of tetrathionate, and amino acid decarboxylases were tested for by using the method of Richard (25) for aerobic gram-negative bacteria. The denitrification test was performed on a medium containing 20 g of casein hydrolysate (Institut Pasteur Production, Paris, France) per liter of distilled water. The same medium without potassium nitrate was used as a control. Growth

with and without gas production was recorded within 48 h. Levan production from sucrose was examined by using the technique of Stolp and Gadkari (31). The accumulation of poly-β-hydroxybutyrate (PHB) was determined in a standard mineral medium (31) supplemented with 0.5% (wt/vol) DL-3-hydroxybutyrate. The presence of PHB was demonstrated by staining with 0.4% (wt/vol) Sudan black B in alcohol at 70°C (representative strains from subclusters A1, A2, B1, and C1 to C5). Acidification or alkalization of the media containing sugars and alcohols was tested in Hugh-Leifson medium (9) and was read after 24 h and 48 h. Assimilation of 150 compounds, including carbohydrates,

FIG. 1. Phenotypic dendrogram based on unweighted pair group average linkage. S_D , Dice similarity index.

organic acids, and amino acids, as sole carbon sources was tested by using API 50 CH, API 50 A0, and API 50 AA kits (API System, La Balme les Grottes, France), respectively. Growth was observed within 1, 2, and 5 days; when no growth was detected on any substrate (strains G 4367 and DSM 50145), amino acids and vitamins (Biomerieux, Lyon, France) were added to the agar medium to final concentrations of 1% (wt/vol).

Numerical analysis. The similarity between strains was calculated by using the Dice index (8). Classification of the strains was based on the unweighted pair group average linkage method (4, 28).

RESULTS

Our dendrogram (Fig. 1) exhibited six main clusters, designated clusters A through F, which could be subdivided, at higher levels of similarity, into the following 20 subclusters: A1, A2, B1, C1 through C5, D1, D2, E1 through E3, and F1 through F7. Two strains did not fall into any cluster.

Clusters A and B. Clusters A and B contained 12 strains each. Subclusters A1, A2, and B1 comprised 7, 3, and 10 strains, respectively; 4 strains belonging to clusters A (2 strains) and B (2 strains) did not fall into any subcluster.

Most of the strains were received as belonging to the species *P. alcaligenes*; exceptions were seven strains received as *P. pseudoalcaligenes* (three strains in subcluster A2, two cluster A strains that did not fall into any subcluster, and two of the strains belonging to subcluster B1) and one strain received as *P. mendocina* (belonging to subcluster B1). Type strain ATCC 14909 of *P. alcaligenes* was in subcluster B1.

Phenotypic descriptions of these groups are presented in Tables 2 and 3. The differentiation of clusters A and B was based on production of arginine dihydrolase and utilization of DL-5-amino-valerate, putrescine, spermine, caprylate, pelargonate, adipate, pimelate, suberate, azelate, sebacate, DL-glycerate, and levulinate. Subclusters A1 and A2 (Table 3) showed clear phenotypic differences since most strains of subcluster A1 utilized itaconate, mesaconate, *m*-hydroxybenzoate, and *p*-hydroxybenzoate, whereas strains of subcluster A2 were unable to utilize these compounds. Conversely, all strains of subcluster A2 were able to grow on glycerol, fructose, L-citrulline, and DL-4-aminobutyrate, whereas strains of subcluster A1 failed to do so.

Formation of PHB inclusions was observed in only 29% of the subcluster A1 strains tested. In subcluster A2, only strain G 4767 was tested, and it contained inclusion bodies. No inclusions were detected in strains ATCC 14909^T (T = type strain) and CCUG 1316 (the only members of subcluster B1 tested).

Cluster C. Cluster C, containing 48 strains, was divided into the following five subclusters: subcluster C1 (2 strains; 1 strain received as *P. alcaligenes* and 1 strain received as *P. pseudoalcaligenes*), subcluster C2 (14 strains received as *P. pseudoalcaligenes*), subcluster C3 (25 strains; 23 strains labeled *P. pseudoalcaligenes*, including type strain ATCC 17440 [= CIP 66-14] and 2 strains labeled *P. alcaligenes*), subcluster C4 (2 strains; 1 strain received as *P. pseudoalcaligenes* and 1 strain received as *Pseudomonas oleovorans*), and subcluster C5 (2 strains labeled *P. pseudoalcaligenes*).

Three strains (strains CCUG 15481, R 76-80, and R 74-80) in cluster C were not classified. Most of the cluster C strains (85 to 100%) were differentiated from cluster A and B strains (Table 2) by the following tests: production of Tween esterase, growth on 5% (wt/vol) NaCl, and assimilation of fructose and L-leucine. Numerous characteristics differentiate subclusters C1 through C5 from each other and from clusters A and B (Table 3).

The presence of PHB could not be demonstrated in strains representative of subclusters C1 through C5 (strains CIP 66-14, CCUG 5181, G 5035, G 4739, and R 3-82).

Cluster D. Cluster D, which contained 13 strains, was divided into two subclusters, subcluster D1 (2 strains; 1 strain received as *P. stutzeri* and 1 strain received as *Pseudomonas* sp.) and subcluster D2 (10 strains received as *P. mendocina*, including type strain ATCC 25411). One cluster D strain (a *P. pseudoalcaligenes* strain) did not fall into either subcluster. All of the strains of cluster D were capable of assimilating glucose, glycine, L-valine, L-serine, L-histidine, betaine, sarcosine, and *trans*-aconitate.

Cluster E. Cluster E, containing 45 strains (all received as *P. stutzeri*), was divided into the following three subclusters: subcluster E1 (3 strains), subcluster E2 (39 strains, including type strain ATCC 17588), and subcluster E3 (2 strains). One strain did not fall into any subcluster. All of the strains of cluster E were easily differentiated from all of the other taxa by their assimilation of maltose, starch, and glycogen (Table

TABLE 2. Characteristics for differentiating clusters A through F

Characteristic	% of strains positive					
	Cluster A (n = 12) ^a	Cluster B (n = 12)	Cluster C (n = 48)	Cluster D (n = 13)	Cluster E (n = 45)	Cluster F (n = 23)
Gelatin hydrolysis	0	0	2	0	0	65
Malonate	0	0	0	69	100	74
Arginine dihydrolase	0	75	63	100	11	96
Tween esterase	58	98	10	92	96	65
Nitrate reduction	75	83	96	8	98	52
Starch hydrolysis	0	0	0	15	91	0
Growth on:						
10% TTC ^b	17	0	0	0	0	74
5% NaCl	0	8	94	100	98	52
Carbon sources						
D-Ribose	0	0	0	0	0	100
Glucose	8	0	6	100	98	96
Mannose	0	0	0	0	4	78
Fructose	33	0	85	85	91	100
Maltose	0	0	2	0	93	0
Starch	0	0	2	0	91	0
Glycogen	0	0	0	0	91	0
Gluconate	83	0	17	100	93	100
2-Ketogluconate	17	0	0	0	0	96
Glycine	0	0	10	100	27	61
L-Leucine	83	92	0	100	96	87
L-Valine	25	58	0	100	96	100
L-Serine	17	17	23	100	2	96
L-Histidine	75	58	29	100	0	96
L-Aspartate	83	92	13	100	71	100
L-Glutamate	92	100	38	100	93	100
Betaine	0	8	4	100	2	96
L-Arginine	0	92	33	92	0	91
β-Alanine	17	75	4	85	0	100
DL-5-Aminovalerate	0	75	6	8	31	100
Sarcosine	0	0	8	100	2	96
Ethanalamine	0	8	69	0	7	91
Putrescine	0	92	73	100	0	100
Spermine	0	83	38	100	0	91
Isovalerate	83	8	0	77	13	74
n-Caproate	75	75	21	92	67	91
Heptanoate	25	83	23	100	91	96
Caprylate	8	92	29	100	93	100
Pelargonate	8	92	44	100	93	100
Adipate	92	0	0	0	2	22
Pimelate	75	0	0	0	0	13
Suberate	100	8	0	0	87	0
Azelate	100	8	0	8	98	22
Sebacate	100	8	0	15	100	22
DL-Glycerate	83	0	94	100	100	100
Levulinate	83	8	15	92	93	70
Aconitate	8	0	2	92	0	57

^a n, Number of strains.^b TTC, 2,3,5-Triphenyltetrazolium chloride.

2). Characteristics for differentiating subclusters E1 through E3 are shown in Table 4.

Cluster F. Cluster F comprised 23 strains which belonged to the following species: *P. putida* (subclusters F2 [2 strains] and F1 [2 strains], corresponding to biovars A and B, respectively), *P. stutzeri* and *P. mendocina* (subcluster F3 [2 strains of each species]), *P. aeruginosa* (subcluster F4 [2 strains]), *P. fluorescens* biovar A (subcluster F5 [2 strains]), *P. aureofaciens* and *P. chlororaphis* (subcluster F6 [1 strain of each species]), and *P. fluorescens* biovar C (subcluster F7 [2 strains]). Seven strains did not fall into any subcluster (*P. fluorescens* biovar G [2 strains], *P. fluorescens* biovar B [2 strains], *P. fluorescens* biovar F [1 strain], *P. mendocina* [1 strain], and *Pseudomonas* sp. [1 strain]).

The tests which differentiated subclusters F1 through F7 are shown in Table 4.

DISCUSSION

Clusters A and B: *P. alcaligenes* and phenotypically similar strains. Most of the strains assigned to the species *P. alcaligenes* were in three subclusters, subclusters A1, A2, and B1. Six strains previously studied by Pickett and Greenwood (21) fell into subclusters A1 (strains G 4519, G 3621, G 3603, and G 3530) and B1 (strains ATCC 14909^T and G 3512). Nutritional reactions (growth on adipate, caprate, caprylate, pimelate, and suberate) which varied among *P. alcaligenes* strains in previous work (22) were useful in differentiating subclusters A1, A2, and B1 (Table 3).

TABLE 3. Characteristics for differentiating subclusters A1, A2, B1, and C1 through C5

Characteristic	% of strains positive							
	Subcluster A1 (n = 7) ^a	Subcluster A2 (n = 3)	Subcluster B1 (n = 10) ^b	Subcluster C1 (n = 2)	Subcluster C2 (n = 14)	Subcluster C3 (n = 25) ^c	Subcluster C4 (n = 2)	Subcluster C5 (n = 2)
Simmons citrate	71	0	80	100	57	20	0	0
Arginine dihydrolase	0	0	100	50	79	64	0	0
RAT-TTR ^d	86	0	0	0	0	0	50	100
Growth on 3% NaCl	100	0	90	100	100	100	100	100
Growth at 42°C	14	0	60	100	100	84	100	100
Carbon sources								
Glycerol	0	100	10	0	50	20	50	0
Glucose	0	33	0	0	0	0	100	0
Fructose	0	100	0	0	93	88	100	50
D-Alanine	57	100	90	0	57	8	0	0
L-Alanine	57	100	100	0	86	12	100	0
L-Serine	0	67	20	0	57	8	50	0
L-Tyrosine	100	33	30	0	86	4	0	0
L-Citrulline	0	100	0	0	0	0	0	0
L-Arginine	0	0	90	0	64	20	100	0
L-Proline	86	100	90	0	86	24	100	0
β-Alanine	0	67	80	0	14	0	0	0
DL-4-Amino-butyrate	0	100	100	0	64	60	0	50
Putrescine	0	0	90	0	93	72	100	50
Acetate	100	67	80	0	86	16	0	0
Propionate	100	67	100	0	100	16	0	0
Butyrate	100	100	90	0	100	44	0	50
n-Valerate	100	67	90	0	71	4	0	0
n-Caproate	100	33	80	0	64	0	0	0
Heptanoate	29	0	90	0	71	0	0	0
Caprylate	0	0	100	0	71	4	100	0
Pelargonate	0	0	100	0	86	20	100	0
Caprate	29	0	100	0	86	36	0	0
Succinate	100	100	90	0	100	100	100	100
Glutarate	100	100	50	0	100	96	100	50
Glycolate	71	0	0	0	0	0	0	0
DL-3-Hydroxybutyrate	100	100	80	0	100	96	100	100
D-Malate	100	67	10	0	79	24	0	0
Itaconate	86	0	0	50	64	92	100	50
Mesaconate	71	0	0	0	64	96	100	100
m-Hydroxybenzoate	86	0	0	0	0	0	0	0
p-Hydroxybenzoate	100	0	0	0	21	8	50	0
Denitrification								
With gas	0	100	60	0	0	0	0	0
Without gas	0	0	10	0	0	0	0	0

^a n, Number of strains.^b The type strain of *P. alcaligenes* is in subcluster B1.^c The type strain of *P. pseudoalcaligenes* is in subcluster C3.^d RAT-TTR, Anaerobic respiration of tetrathionate-tetrathionate reductase.

Attention has been drawn to the problem of phenotypic differentiation of *P. alcaligenes* from *Pseudomonas testosteroni* (21, 22). The latter species and *Pseudomonas acidovorans* have been reclassified in the genus *Comamonas* (6) as *Comamonas testosteroni* and *Comamonas acidovorans* (33), respectively, and it is necessary to be able to separate them from *P. alcaligenes* and, in particular, from subcluster A1. The PHB inclusions present in almost all *P. testosteroni* strains (21) and the assimilation of glycine, L-valine, DL-kynurenine, trans-aconitate, and citrate exhibited by more than 80% of the strains of this species (18) were not observed in strains of subcluster A1.

Although strain G 4767 (subcluster A2) contained PHB inclusions, discrimination of the three strains of subcluster A2 from *P. testosteroni* was facilitated by the following nutritional characteristics: assimilation of glycine, L-valine, glycerol, sorbitol, L-citrulline, glycolate, itaconate, trans-aconitate, and citrate. Only subcluster B1, which included the type strain of the species, could be considered *P.*

alcaligenes sensu stricto. The DNAs of two *P. alcaligenes* strains studied by Ralston-Barrett et al. (24) were found to be 76 and 56% related to the type strain. This difference confirmed the heterogeneity shown by the phenotypic results of these authors (21, 22).

The definition of the species *P. alcaligenes* given by Palleroni (18) could be amended in the following features (the percentages of strains of subcluster B1 yielding positive results are given in parentheses): utilization of L-aspartate (92%), β-alanine (90%), putrescine (90%), and DL-3-hydroxybutyrate (80%). The converse results were presented by Palleroni (18) for these nutritional characteristics, except for assimilation of β-alanine and putrescine, for which positive or negative reactions could occur.

The relationships among subclusters A1, A2, and B1 and between subcluster A1 and *P. testosteroni* will be clarified after DNA-DNA hybridization experiments. The high number of phenotypic criteria which differentiated subclusters A1 and A2 from subcluster B1 (Tables 2 and 3) and from *C.*

TABLE 4. Characteristics for differentiation of subclusters D1, D2, and E1 through E3

Characteristic	% of strains positive				
	Subcluster D1 (n = 2) ^a	Subcluster D2 (n = 10) ^b	Subcluster E1 (n = 3)	Subcluster E2 (n = 39) ^c	Subcluster E3 (n = 2)
Growth at 42°C	0	90	0	87	0
Urease	0	50	0	3	0
Gelatinase	0	0	0	0	0
Malonate	100	70	100	100	100
Tween esterase	100	100	100	97	0
RAT-TTR ^d	0	0	33	28	0
Nitrate reduction	0	0	100	97	100
Tributylin	100	0	0	69	0
Levan from sucrose	100	0	0	0	0
Growth on 10% TTC ^e	0	0	0	0	0
Growth on 5% NaCl	100	100	100	97	100
Carbon sources					
Mannose	0	0	0	5	100
Mannitol	100	0	0	87	100
L-Leucine	100	100	100	95	0
L-Isoleucine	50	50	0	92	0
DL-4-Aminobutyrate	50	80	0	74	0
Histamine	50	0	0	0	0
Tryptamine	0	100	0	0	0
Isobutyrate	0	100	67	92	0
Isovalerate	0	90	0	15	0
n-Caproate	100	100	0	74	50
Malonate	100	60	100	100	100
Suberate	0	0	67	85	50
Azelate	0	0	100	97	100
Sebacate	50	0	100	100	100
Glycolate	100	90	100	100	100
D-Malate	100	100	0	85	0
meso-Tartrate	100	0	0	0	0
Itaconate	100	100	100	97	100
Mesaconate	100	100	100	100	100
Benzoate	0	100	33	82	50
Alkalinization of:					
Melibiose	0	90	67	20	0
Mannitol	0	100	100	13	0

^a n, Number of strains.^b The type strain of *P. mendocina* is in subcluster D2.^c The type strain of *P. stutzeri* is in subcluster E2.^d RAT-TTR, Anaerobic respiration of tetrathionate-tetrathionate reductase.^e TTC, 2,3,5-Triphenyltetrazolium chloride.

testosteroni suggests that these phena could represent one or two new species.

Cluster C: *P. pseudoalcaligenes* and related strains. Cluster C contained most of the strains named *P. pseudoalcaligenes*. Six strains from the study of Stanier et al. (30), whose DNAs had been found to be 79 to 82% related to the DNA of the type strain of *P. pseudoalcaligenes* (24), fell into subcluster C3 (Stanier strains 63^T, 65, 66, 299, and 417) and into cluster A (Stanier strain 297, not a member of either subcluster A1 or subcluster A2). Because of the position of these strains in subcluster C3, which included the type strain, the species *P. pseudoalcaligenes* could be described by the phenotypic characteristics of this subcluster. However, subcluster C2 exhibited characteristics more similar to those described by Stanier et al. (30), Ralston-Barrett et al. (24), and Palleroni (18) for this species than subcluster C3 did (Table 3). The discrepancy between the results for nutritional characteristics observed in our study and the results described previously cannot be explained at the present time.

Subclusters C1, C4, and C5, which contained two strains each, could be separated on the basis of the nutritional characteristics shown in Table 3. Subcluster C4 contained the type strain of *P. oleovorans*, strain CCUG 2087 (=

ATCC 8062). This species was included in Section V of the genus *Pseudomonas* by Palleroni (18). Although the name *P. oleovorans* was included on the Approved Lists of Bacterial Names (27), the type strain is maintained in the Culture Collection, University of Göteborg, Göteborg, Sweden (from which we received it), under the name *P. pseudoalcaligenes*. The close relationship of *P. oleovorans* to *P. pseudoalcaligenes* indicated in this analysis should be confirmed by DNA-DNA hybridization.

Cluster D: *P. mendocina* and related strains. The 10 strains comprising subcluster D2 were all received as *P. mendocina* (and included the type strain). The phenotypic characteristics which we determined agreed with most of the previously published definitions of this species (9, 11, 18, 19; G. L. Gilardi, personal communication), with only a few exceptions. We could not demonstrate positive urease reactions in any of our strains, whereas positive reactions were reported for 100 and 50% of the strains studied by one of us (11) and by Gilardi (personal communication), respectively. Negative results were also obtained for reduction of nitrate and reduction of nitrite; these tests differentiated cluster D from clusters A, B, C, and E. Most strains (80%) of subcluster D2 exhibited anaerobic growth on nitrate medium, when nitrate

was used as the electron acceptor (denitrification test). This characteristic was also noted by Palleroni (18). One of us (11) reported that his strains produced opalescence on lecithovitin agar and all grew on cetrimide agar. The strains included in our analysis did not give an egg yolk reaction characterized by an opaque precipitate. Gilardi (9) found that none of his strains grew on cetrimide, but later data (Gilardi, personal communication) showed that some of his strains were able to grow on this medium.

Cluster D2 closely matched the description of *P. mendocina* presented by Palleroni (18) based on the assimilation pattern for most carbon substrates. The only exceptions were glycine, tryptamine, isobutyrate, D-malate, and benzoate, which were used by all of the strains in this study, whereas Palleroni (18) found that different strains gave different results. Also, a negative reaction was observed for DL-4-aminobutyrate by Palleroni, whereas 80% of the strains in our study gave positive results.

The two strains of subcluster D1 were not received as *P. mendocina* but were phenotypically similar to this species; they differed from strains of subcluster D2 only in the following reactions: no growth at 42°C, production of lipase from tributyrin, utilization of mannitol and *m*-tartrate as sole carbon sources, and no assimilation of tryptamin, isobutyrate, or benzoate.

Cluster E: *P. stutzeri* and related strains. A total of 45 strains comprised cluster E, and subcluster E2 was the largest subcluster (39 strains); the latter contained the type strain of *P. stutzeri*, strain ATCC 17588. The nutritional characteristics of this species were reported by Stanier et al. (30) and were also given by Palleroni (18). Other characteristics were described by Gilardi (9; Gilardi, personal communication) and by one of us (11), who discussed previous definitions of the species (1, 12, 23, 32).

Most of the characteristics of subcluster E2 agreed with previously published definitions of *P. stutzeri* (9, 11, 18, 30; Gilardi, personal communication), especially the ability to denitrify and assimilation of maltose and starch as sole carbon sources, which were positive for almost all strains of cluster E. Growth on cetrimide was reported as positive for 54% of the strains studied by one of us (11), whereas Gilardi (9) did not observe growth of any of his strains on this medium (9; Gilardi, personal communication); 16% of the strains of our subcluster E2 did give positive results in this test. Further discrepancies were noticed in the following tests: arginine dihydrolase, egg yolk reaction, and phenylalanine deaminase. Most of the strains were negative for arginine dihydrolase and the egg yolk reaction in this study, in agreement with Gilardi (9), but not with one of us (11), who observed that 48 and 86% of the strains tested were positive in these tests, respectively. The percentage of phenylalanine deaminase-positive strains was very high in our analysis, especially in subcluster E2 (97% of the strains were positive). The following proportions of strains have been reported to give positive results in this test: 37% (9), 54% (Gilardi, personal communication), and 10% (11). Most of the nutritional characteristics studied in this analysis agreed with those given by Stanier et al. (30) or Palleroni et al. (19) for *P. stutzeri*. A small number of discrepancies concerned subcluster E2. The strains of *P. stutzeri* studied by Stanier et al. (30) were able to utilize citraconate (80% of the strains), sucrose (100% of the strains), putrescine (70% of the strains), and aconitate (70% of the strains); however, the strains studied by Palleroni (18) could not use citraconate or sucrose, nor could those in our analysis. Palleroni (18) did find that 76 and 78% of his strains utilized putrescine and

trans-aconitate, respectively, whereas these carbon sources were not assimilated by any of the strains in our study. Suberate, levulinate, isobutyrate, and DL-4-amino-butyrate were used by more than 70% of the strains in our study (87, 93, 92, and 74%, respectively), but not by the strains studied by Palleroni (18). All of the strains of cluster E were able to use propionate as a sole carbon source. This test was the only nutritional characteristic found by Stanier et al. (30) which showed some ability to differentiate the two *P. stutzeri* groups separated on the basis of guanine-plus-cytosine content (30).

The strains of subclusters E1 and E3 were slightly different from those of subcluster E2. These subclusters could be new taxa and could explain some of the variation apparent in previously published definitions, such as absence of growth at 42°C (subclusters E1 and E3), absence of Tween esterase (subcluster E3), and absence of growth on L-leucine (subcluster E3), L-isoleucine (subclusters E1 and E3), DL-4-amino-butyrate (subclusters E1 and E3), isobutyrate (subclusters E1 and E3), D-malate (subclusters E1 and E3), and mannose (subcluster E3).

Cluster F: fluorescent strains. Cluster F contained seven subclusters (subclusters F1 through F7). Our aim was not to study the fluorescent *Pseudomonas* species in detail, so the number of strains included was very small, and these organisms only served as controls. However, it was interesting to note the separation of *P. putida* biovars A (subcluster F2) and B (subcluster F1), of *P. fluorescens* biovars A (subcluster F5) and C (subcluster F7), and of *P. aeruginosa* (subcluster F4). The species *P. aureofaciens* and *P. chlororaphis* were contained in the same subcluster (subcluster F6). A single taxon, which included both species, was also found by Mölin and Ternström (15) in their fluorescent supercluster and by Sneath et al. (29), who performed a numerical analysis on the phenotypic data of Stanier et al. (30). Strains of *P. fluorescens* biovars B, G, and F remained ungrouped, but this observation was not surprising because of the low number of strains included in the analysis.

The strains of subcluster F3, which were received as *P. stutzeri* or *P. mendocina*, shared a fluorescent pigment with all of the other strains of cluster F but were well separated from them. This raises the following question: could they belong to the species *Pseudomonas fragi* or *Pseudomonas lundensis*. The latter species were recently studied by Mölin and Ternström (15); some of the strains of these authors exhibited a fluorescent pigment. The subcluster F3 strains shared with strains of *P. fragi* and *P. lundensis* the ability to assimilate phenylacetate, valerate, citrulline, and methionine (15). In contrast, however, the subcluster F3 strains could not utilize D-xylose, D-arabinose, or L-arabinose.

In conclusion, phenotypic criteria, especially nutritional characteristics, can differentiate groups which might be confirmed as being genospecies in most cases. Some difficulties must be resolved in the separation of some species, such as *P. alcaligenes* and *C. testosteroni*. In these cases, new tests will be necessary, but it is obvious that criteria such as number of flagella or presence of PHB inclusion bodies would be unsuitable for routine identification purposes. Furthermore, it appears from this analysis that alkalization or oxidation of various substrates does not provide any information for classification purposes.

Our analysis permits revised phenotypic definitions of *P. alcaligenes*, *P. pseudoalcaligenes*, *P. stutzeri*, and *P. mendocina*. The subclusters which do not contain any type strains (subclusters A1, A2, C1, C2, C4, C5, D1, and E3) will be studied by using the DNA-DNA hybridization procedure

to determine their precise taxonomic positions. We agree with Mölin and Ternström (15) that the number of species included in *Pseudomonas* ribosomal ribonucleic acid group 1 may well be extended, and in the future this group might be subdivided into several genera. In view of the data presented by various authors (15, 16, 34) and from our analysis, a good revision of this group can be expected.

LITERATURE CITED

- Alexander, J. J., and J. F. Lewis. 1976. Pitting of agar surface by *Pseudomonas stutzeri*. J. Clin. Microbiol. 3:381.
- Banks, J. G., and R. G. Board. 1983. The classification of pseudomonads and other obligately aerobic Gram-negative bacteria from British pork sausage and ingredients. Syst. Appl. Microbiol. 4:424-438.
- Champion, A. B., E. L. Barrett, N. J. Palleroni, K. L. Soderberg, R. Kunisawa, R. Contopoulou, A. C. Wilson, and M. Doudoroff. 1980. Evolution in *Pseudomonas fluorescens*. J. Gen. Microbiol. 120:485-511.
- Delabre, M., A. Bianchi, and M. Veron. 1973. Etude critique des méthodes de taxonomie numérique. Application à une classification des bactéries aquicoles. Ann. Microbiol. (Paris) 124A: 489-506.
- De Vos, P., and J. De Ley. 1983. Intra- and intergeneric similarities of *Pseudomonas* and *Xanthomonas* ribosomal ribonucleic acid cistrons. Int. J. Syst. Bacteriol. 33:487-509.
- De Vos, P., K. Kersters, E. Falsen, B. Pot, M. Gillis, P. Segers, and J. De Ley. 1985. *Comamonas* Davis and Park 1962 gen. nov., nom. rev. emend., and *Comamonas terrigena* Hugh 1962 sp. nov., nom. rev. Int. J. Syst. Bacteriol. 35:443-453.
- Gavini, F., C. Ferragut, B. Lefebvre, and H. Leclerc. 1976. Etude taxonomique d'entérobactéries appartenant ou apparentées au genre *Enterobacter*. Ann. Microbiol. (Paris) 127B: 317-335.
- Gavini, F., B. Lefebvre, and H. Leclerc. 1976. Positions taxonomiques d'entérobactéries H₂S⁻ par rapport au genre *Citrobacter*. Ann. Microbiol. (Paris) 127A:275-295.
- Gilardi, G. L. (ed.). 1978. Glucose non-fermenting Gram-negative bacteria in clinical microbiology. CRC Press, Inc., Boca Raton, Fla.
- Goto, M. 1983. *Pseudomonas pseudoalcaligenes* subsp. *konjaci* subsp. nov., the causal agent of bacterial leaf blight of Konjac (*Amorphophalus konjac* Koch.). Int. J. Syst. Bacteriol. 33: 539-545.
- Holmes, B. 1986. Identification and distribution of *Pseudomonas stutzeri* in clinical material. J. Appl. Bacteriol. 60:401-411.
- Lapage, S. P., L. R. Hill, and J. D. Reeve. 1968. *Pseudomonas stutzeri* in pathological material. J. Med. Microbiol. 1:195-202.
- Lefebvre, B., and F. Gavini. 1982. Theory and programming of a computer identification system for coliform strains. J. Appl. Bacteriol. 52:325-328.
- Mölin, G., and A. Ternström. 1982. Numerical taxonomy of psychrotrophic pseudomonads. J. Gen. Microbiol. 128:1249-1264.
- Mölin, G., and A. Ternström. 1986. Phenotypically based taxonomy of psychrotrophic *Pseudomonas* isolated from spoiled meat, water, and soil. Int. J. Syst. Bacteriol. 36:257-274.
- Mölin, G., A. Ternström, and J. Ursing. 1986. *Pseudomonas lundensis*, a new bacterial species isolated from meat. Int. J. Syst. Bacteriol. 36:339-342.
- Palleroni, N. J. 1981. Introduction to the family *Pseudomonadaceae*, p. 655-665. In M. P. Starr, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes. A handbook on habitats, isolation, and identification of bacteria. Springer-Verlag, Berlin.
- Palleroni, N. J. 1984. *Pseudomonadaceae*, p. 141-199. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
- Palleroni, N. J., M. Doudoroff, R. Y. Stanier, R. E. Solanes, and M. Mandel. 1970. Taxonomy of the aerobic pseudomonads: the properties of the *Pseudomonas stutzeri* group. J. Gen. Microbiol. 60:215-231.
- Palleroni, N. J., R. Kunisawa, R. Contopoulou, and M. Doudoroff. 1973. Nucleic acid homologies in the genus *Pseudomonas*. Int. J. Syst. Bacteriol. 23:333-339.
- Pickett, M. J., and R. Greenwood. 1986. *Pseudomonas alcaligenes* and *Pseudomonas testosteroni*: characterization and identification. Curr. Microbiol. 13:197-201.
- Pickett, M. J., and J. R. Greenwood. 1986. Identification of oxidase-positive, glucose-negative, motile species of nonfermentative bacilli. J. Clin. Microbiol. 23:920-923.
- Pickett, M. J., and M. M. Pedersen. 1970. Characterization of saccharolytic non-fermentative bacteria associated with man. Can. J. Microbiol. 16:351-362.
- Ralston-Barrett, E., N. J. Palleroni, and M. Doudoroff. 1976. Phenotypic characterization and deoxyribonucleic acid homologies of the "*Pseudomonas alcaligenes*" group. Int. J. Syst. Bacteriol. 26:421-426.
- Richard, C. 1978. Techniques de recherche d'enzymes utiles au diagnostic de bactéries à Gram négatif. Ann. Biol. Clin. 36: 407-424.
- Shaw, B. G., and J. B. Latty. 1982. A numerical taxonomic study of *Pseudomonas* strains from spoiled meat. J. Appl. Bacteriol. 52:219-228.
- 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.
- Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. W. H. Freeman, San Francisco.
- Sneath, P. H. A., M. Stevens, and M. J. Sackin. 1981. Numerical taxonomy of *Pseudomonas* based on published records of substrate utilization. Antonie van Leeuwenhoek J. Microbiol. Serol. 47:423-448.
- Stanier, R. Y., N. J. Palleroni, and M. Doudoroff. 1966. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. 43:159-271.
- Stolp, H., and D. Gadkari. 1981. Non-pathogenic members of the genus *Pseudomonas*, p. 719-741. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes. A handbook on habitats, isolation, and identification of bacteria. Springer-Verlag, Berlin.
- Sutter, V. L. 1968. Identification of *Pseudomonas* species isolated from hospital environment and human sources. Appl. Microbiol. 16:1532-1538.
- Tamaoka, J., D.-M. Ha, and K. Komagata. 1987. Reclassification of *Pseudomonas acidovorans* den Dooren de Jong 1926 and *Pseudomonas testosteroni* Marcus and Tlalalay 1956 as *Comamonas acidovorans* comb. nov. and *Comamonas testosteroni* comb. nov., with an emended description of the genus *Comamonas*. Int. J. Syst. Bacteriol. 37:52-59.
- Ursing, J. 1986. Similarities of genome deoxyribonucleic acids of *Pseudomonas* strains isolated from meat. Curr. Microbiol. 13:7-10.
- Woese, C. R., P. Blanz, and C. M. Hahn. 1984. What isn't a pseudomonad: the importance of nomenclature in bacterial classification. Syst. Appl. Microbiol. 5:179-195.