INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Oct. 1996, p. 1138–1144 Vol. 46, No. 4
0020-7713/96/$04.00+0
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DNA Relatedness among *Pseudomonas* Strains Isolated from Natural Mineral Waters and Proposal of *Pseudomonas veronii* sp. nov.

MALIKA ELOMARI,[1] LOÏC COROLER,[1] BART HOSTE,[2] MONIQUE GILLIS,[2] DANIEL IZARD,[1] AND HENRI LECLERC[1]*

Laboratoire de Bactériologie-Hygiène, Faculté de Médecine Henri Warembourg, 59045 Lille Cedex, France,[1] and Laboratorium voor Microbiologie, Universiteit Ghent, B-9000 Ghent, Belgium[2]

The taxonomic position of eight strains isolated from mineral water and previously grouped in the authentic pseudomonads on the basis of a phenotypic analysis (cluster Ib of M. Elomari, L. Coroler, D. Izard, and H. Leclerc [J. Appl. Bacteriol. 78:71–81, 1995]) has been further studied by DNA-DNA hybridizations. Using the S1 nuclease method at 60°C and labeled reference DNA from a representative strain, CFML 92-134, we showed that members of cluster Ib constituted a homogeneous group with a relative binding ratio of greater than 80% and changes in melting temperature of less than 1°C. With a total of 67 strains representing known or partially characterized species of the genus *Pseudomonas*, only 4 to 47% DNA hybridization and changes in melting temperature of between 8 and 20°C were found, the highest hybridization values being measured with members of the saprophytic fluorescent pseudomonads. Since cluster Ib could also be clearly differentiated from members of the latter group and from other phenotypic clusters containing isolates from mineral water, we designated the Ib strains members of a new *Pseudomonas* species for which the name *Pseudomonas veronii* sp. nov. has been proposed. Members of this species grew on α-aminobutyrate, sucrose, butyrate, isobutyrate, erythritol, L-tryptophan, and trigonelline as sole sources of carbon and energy. The average G+C content of the DNA of the eight strains of *P. veronii* was 61.5 ± 0.5 mol%. The type strain is CFML 92-134^T (CIP 104663^T), with a G+C content of 61 mol%. The clinical significance of *P. veronii* is unknown.

Pseudomonadaceae is a very large and important family of gram-negative bacteria including the genera *Pseudomonas*, *Xanthomonas*, *Zoogloea*, and *Frateuria* (32, 34). The genus *Pseudomonas* is particularly interesting, because *Pseudomonas* strains are important not only medically, as opportunistic pathogens that cause disease in animals and humans (33), but also environmentally and agriculturally, since many species are phytopathogens (18). It has been recognized that the members of this taxon as described by Palleroni et al. (38) are phylogenetically too heterogeneous to be considered a single genus. At least five groups have been recognized on the basis of DNA-rRNA hybridizations, and some of these groups are as distantly related to each other as they are to *Escherichia coli* (32). *Pseudomonas* rRNA group I (32) is part of rRNA superfamily II (11) or the gamma subclass of the *Proteobacteria* (40, 46), where it constitutes a separate rRNA complex (11–13, 41, 51). This group represents the authentic pseudomonads containing the type species *Pseudomonas aeruginosa* (32). Consequently, the members of the other four rRNA groups belonging in the beta and gamma subclasses have been classified in other appropriate existing genera or in newly created genera such as *Stenotrophomonas* (36), *Comamonas* (13, 48), *Acidovorax* (52), *Hydrogenophaga* (50), *Sphingomonas* (55), *Burkholderia* (53), *Ralstonia* (54), *Telluria* (5), and the recently proposed genus *Brevundimonas* (44).

The genus *Pseudomonas* sensu stricto includes saprophytic and phytopathogenic fluorescent pseudomonads, nonpigmented denitrifying strains of the *P. stutzeri* group (37), and nonpigmented strains that constitute the *P. alcaligenes* group (39). The saprophytic species of fluorescent pseudomonads are characterized by the production of water-soluble pigments (pyoverdins) and can be distinguished from the phytopathogenic species by their positive arginine dihydrolase reaction, their more rapid growth in most media, and their ability to utilize certain substrates. The complexity of the fluorescent saprophytes other than *P. aeruginosa* (considered a homogeneous species [32]) has been well illustrated by extensive studies (2, 7, 23, 29, 30, 35, 47). In total, 81 biovars were recognized by Jessen (23). This number was reduced to seven *P. fluorescens* biovars and two biovars of *P. putida* by Stanier et al. (47). Champion et al. (7) defined the relationships among strains of fluorescent pseudomonads by their phenotypic properties, DNA-DNA hybridization, and quantitative microcomplement fixation studies. Five biovars in *P. fluorescens* are now recognized by Palleroni (32), since two biovars previously recognized

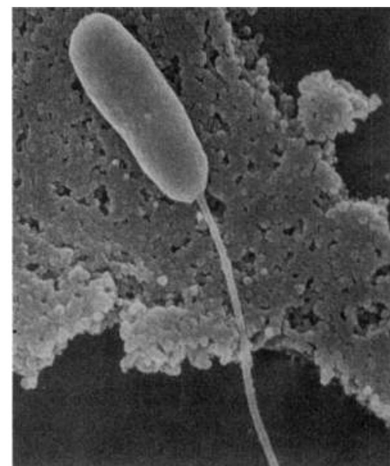

FIG. 1. Electron micrograph of a cell of *P. veronii* CFML 92-134^T showing the single polar flagellum. Magnification, ×20,000.

* Corresponding author. Mailing address: Laboratoire de Bactériologie-Hygiène, Faculté de Médecine Henri Warembourg, 1 place de Verdun, 59045 Lille Cedex, France. Phone: (33) 03 20 44 45 97. Fax: (33) 03 20 52 93 61. Electronic mail address: simonet@pop.univ-lille2.fr.

TABLE 1. G+C content of eight *P. veronii* strains and levels of DNA-DNA hybridization with members of clusters IIa and V

Phenotypic cluster or subcluster[a]	Strain[b]	Origin of mineral water[c]	Labeled DNA from strain: CFML 92-134^{T}		CFML 92-111^{T}		G+C content (mol%)
			RBR[d] (%)	ΔT_m (°C)	RBR (%)	ΔT_m (°C)	
Ib	CFML 92-134^{T}	A (1) 1	100		23		61
	CFML 92-140	A (4) 2	98		38		61
	CFML 92-123	B (3) 1	96		53		61
	CFML 92-136	A (2) 1	93		45		62
	CFML 92-143	A (4) 1	90		51		61
	CFML 92-124	B (4) 1	89	0	45		61
	CFML 92-138	A (3) 1	87	0.5	56		61
	CFML 92-133	A (1) 2	80	1	47		61
IIa1[e]	CFML 92-111^{T}	D (3) 3	31		100		
	CFML 92-108	D (2) 3	39		94		
	CFML 92-113	D (4) 3	56		93		
	CFML 92-112	D (2) 2	35		92		
	CFML 92-103	D (4) 1	47		92	0	
	CFML 92-104	D (1) 1	36		87	0	
	CFML 92-102	D (3) 1	43		84	2	
IIa2	CFML 92-116	B (1) 2	20		32		
	CFML 92-126	B (2) 2	33		31		
	CFML 92-120	B (1) 1	32		29		
	CFML 92-125	B (3) 2	42		28		
	CFML 92-115	C (2) 1	29		27		
	CFML 92-119	B (1) 3	37		25		
	CFML 92-122	C (2) 1	24		21		
V	CFML 92-101	D (2) 1	41		33		
	CFML 92-114	C (2) 1	39		43		
	CFML 92-142	A (4) 1	38		40		
	CFML 92-144	A (2) 3	38		31		
	CFML 92-131	A (1) 1	33		15		
	CFML 92-107	D (3) 3	32		48		

[a] IIa could be divided into two subclusters, IIa1 and IIa2, on genomic grounds.
[b] CFML, Collection de la Faculté de Médecine, Lille, France.
[c] All strains were isolated from four French natural mineral waters sources, called A, B, C, and D (Contrexeville [Pavillon spring], Volvic [Clairvic spring], Vittel [Grande source], and Evian, [Cachat spring], respectively) at the point of emergence (1), in the pipeline (2), before bottling (3), and after bottling (4). For each brand, samples were examined once a year, at time 1, 2, or 3.
[d] RBR, relative binding ratio.
[e] The subcluster IIa1 is described as *P. rhodesiae* sp. nov. (8).

appeared so distinct that they were separated under their original species names, *P. chlororaphis* and *P. aureofaciens* (35). Both species were later unified in *P. chlororaphis* (24). Palleroni (32) recognized two biovars in *P. putida*. Barrett et al. (2) described two new biovars: biovar VI of *P. fluorescens* and biovar C of *P. putida*. All these studies emphasized the extreme heterogeneity of fluorescent saprophytic members of the genus *Pseudomonas*.

Nevertheless, pseudomonad identification at the species level continues to be a difficult task, especially for environmental isolates from aquatic ecosystems where pseudomonads are in the majority. Natural mineral waters can be characterized by their bacterial flora and their chemical and physical composition, which are considered indicators of natural and original qualities of the water (1, 43). These bacteria, initially present in very small numbers (less than 10 CFU/ml), multiply normally to reach, within a few days, levels of 10^3 to 10^5 CFU/ml, depending on intrinsic and extrinsic conditions (4, 6). The main factors determining the growth of bacteria in bottled water are the mineral composition of this water, the nature and level of the organic matter, the dissolved oxygen concentration, and the temperature of storage. These factors have been studied by several workers (4, 6, 15, 16, 25, 28, 42, 43). Nutrient concentrations, although low, are sufficient to permit the slow growth of some bacteria initially present in the spring (31, 49). Studies of the bacterial flora of mineral waters have shown that these bacteria are exclusively heterotrophic, oxidative, and gram negative. Our group has also demonstrated that approximately 80% of strains isolated from natural mineral waters were not identifiable and that the majority of strains which could be identified were fluorescent members of the genus *Pseudomonas* (20, 21).

In a previous numerical taxonomy study (17), we provided evidence for the existence of three new phenotypic clusters (Ib, IIa, and V) among strains isolated from natural mineral waters; phenotypically they are related to the fluorescent species of the genus *Pseudomonas*. Clusters Ib and IIa included only natural mineral water strains. Cluster V contained 13 mineral water strains and three culture collection strains of *P. fluorescens* biovar III. In this report we propose a new species, *P. veronii* sp. nov., for the organisms of cluster Ib on the basis of DNA-DNA hybridization and thermal stability of the DNA-DNA hybrids. The type strain is *P. veronii* CFML 92-134^{T} (CIP 104663^{T}).

TABLE 2. Levels of DNA-DNA hybridization between labeled DNA of *P. veronii* CFML 92-134^{T} and various strains of *Pseudomonas* species of section I and V (33)

Source of unlabeled DNA[a]	Binding (%)[b]	ΔT_m (°C)
P. aeruginosa ATCC 10145^{T}	35	20
P. aeruginosa ATCC 27853	16	ND[c]
P. aeruginosa ATCC 15692	8	ND
P. fluorescens biovar I ATCC 13525^{T}	37	16
P. fluorescens biovar I ATCC 17563	39	ND
P. fluorescens biovar I ATCC 17397	18	ND
P. fluorescens biovar II ATCC 17816	47	9
P. fluorescens biovar II DSMZ 50106	42	ND
P. fluorescens biovar II ATCC 17815	41	ND
P. fluorescens biovar II ATCC 17482	40	ND
P. fluorescens biovar III ATCC 17559	33	ND
P. fluorescens biovar III ATCC 17400	31	ND
P. fluorescens biovar III ATCC 17571	14	ND
P. fluorescens biovar IV DSMZ 50415	30	ND
P. fluorescens biovar IV ATCC 12983	19	ND
P. fluorescens biovar V ATCC 14150	44	12
P. fluorescens biovar V ATCC 17518	40	ND
P. fluorescens biovar V ATCC 15916	38	8
P. fluorescens biovar V ATCC 17386	26	ND
P. fluorescens biovar V DSMZ 50148	24	ND
P. fluorescens biovar V ATCC 17573	19	ND
P. marginalis ATCC 10844^{T}	36	11
P. marginalis DSMZ 50275	44	17
P. marginalis DSMZ 50276	35	ND
P. chlororaphis DSMZ 50083^{T}	37	ND
P. chlororaphis ATCC 9447	37	ND
P. chlororaphis ATCC 17414	28	ND
P. aureofaciens CCEB 518^{T}	34	18
P. aureofaciens ATCC 17415	37	ND
P. putida biovar A ATCC 12633^{T}	22	17
P. putida biovar A CFML 90-57	25	ND
P. putida biovar A CFML 90-46	21	ND
P. putida biovar A CFML 90-40	21	ND
P. putida biovar A DSMZ 50208	20	ND
P. putida biovar A CFML 90-39	20	ND
P. putida biovar A CFML 90-47	18	ND
P. putida biovar A CFML 90-52	15	ND
P. putida biovar A CFML 90-139	9	ND
P. putida biovar A CFML 90-42	9	ND
P. putida biovar A CFML 90-49	7	ND
P. putida biovar B ATCC 17484	32	
P. putida biovar B ATCC 17430	28	
P. putida biovar B CCUG 1317	25	
P. lundensis CCM 573^{T}	18	
P. lundensis CCUG 18758	19	
P. syringae ATCC 19310^{T}	27	
P. savastanoi CFBP 1670^{T}	31	
P. savastanoi CFBP 2088	39	
P. savastanoi CFBP 1838	36	
P. viridiflava ATCC 13223^{T}	37	
P. cichorii DSMZ 50259^{T}	16	
P. agarici ATCC 25941^{T}	14	
P. asplenii ATCC 23835^{T}	21	
P. caricapapayae NCPPB 1873^{T}	9	
P. tolaasii NCPPB 2192^{T}	35	
P. tolaasii NCPPB 1616	43	
P. stutzeri ATCC 17588^{T}	7	
P. stutzeri ATCC 17591	8	
P. stutzeri ATCC 17587	7	
P. stutzeri ATCC 17686	6	
P. mendocina ATCC 25411^{T}	4	
P. mendocina ATCC 25412	14	
P. alcaligenes ATCC 14909^{T}	11	
P. pseudoalcaligenes ATCC 17440^{T}	5	
P. pseudoalcaligenes ATCC 12815	6	
P. fragi ATCC 4973^{T}	14	
P. fragi ATCC 27362	18	

Continued

TABLE 2—*Continued*

[a] Abbreviations and locations of culture collections: ATCC, American Type Culture Collection, Rockville, Md.; CCEB, Culture Collection of Entomogenous Bacteria, Institute of Entomology, Czechoslovakia Academy of Sciences, Prague 6, Czech Republic; CCM, Czechoslovak Collection of Microorganisms, J. E. Purkyně University, Brno, Czech Republic; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; CFML, see Table 1; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-38124 Braunschweig, Germany; NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen AB9 8DG, Scotland; NCPPB, National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory, Harpeaden, England; and PDDCC, Culture Collection of Plant Diseases Division, New Zealand Department of Scientific and Industrial Research, Auckland, New Zealand.

[b] Level of relative binding with labeled DNA from strain CFML 92-134^{T} at 60°C.

[c] ND, not determined.

MATERIALS AND METHODS

Bacterial strains. Ninety-five strains previously listed in detail (17) were included in this study. These organisms comprised 28 wild strains isolated from four French natural mineral water sources (A, B, C, and D) (Contrexeville [Pavillon spring], Volvic [Clairvic spring], Vittel [Grande source], and Evian [Cachat spring], respectively) at different sites (see Table 1) and identified as fluorescent pseudomonads) according to the criteria of Palleroni (32), and 67 (type and collection) strains included for control purposes (see Table 2). They represent 13 *Pseudomonas* species (*P. aeruginosa*, *P. fluorescens* biovars I to V, *P. marginalis*, *P. chlororaphis*, *P. aureofaciens*, *P. putida* biovars A and B, *P. syringae*, *P. viridiflava*, *P. cichorii*, *P. stutzeri*, *P. mendocina*, *P. alcaligenes*, and *P. pseudoalcaligenes*) belonging to section I of the genus *Pseudomonas* as described by Palleroni (32); the psychrotrophic species of importance to food microbiology, *P. fragi* and *P. lundensis* (29, 30); the fluorescent organisms isolated from plants and mushrooms of Palleroni's section V (34), *P. agarici*, *P. asplenii*, *P. caricapapayae*, and *P. tolaasii*; and *P. savastanoi*, a recently described species among the pathovar strains of *P. syringae* (18).

Flagellation study. The flagellation of the bacteria was investigated with an electron microscope by performing a negative-staining technique (22) on fixed organisms. The bacteria were suspended in 0.5% (wt/vol) formaldehyde (neutral) and mixed with an equal volume of 2% (wt/vol) potassium phosphotungstate as described previously (22). The stained bacteria were examined with a Japan Electronic Optical Laboratory type 100 CX transmission electron microscope.

DNA preparation and DNA-DNA hybridization. Strains were grown on Mueller-Hinton medium plates. Chromosomal DNA was extracted, purified, and sheared as described previously (3, 26). Native DNA was labeled in vitro with [^{3}H]cytosine by nick translation (19). The procedure used for the hybridization experiments (the S1 nuclease-trichloroacetic acid method) has been previously described (9, 19).

Thermal stability of reassociated DNAs. The temperature at which 50% of reassociated DNA became hydrolyzable by the S1 enzyme (melting temperature [T_m]) was determined by using the method of Crosa et al. (9). The ΔT_m was the difference between the T_m of the heteroduplex and T_m of the homoduplex.

DNA base composition. The G+C contents of DNAs were calculated from T_ms (27) by the equation of De Ley (10).

TABLE 3. Features differentiating between *P. veronii*, *Pseudomonas rhodesiae*, and saprophytic, fluorescent species and biovars of *Pseudomonas* section I (38)

Characteristic	*P. veronii* sp. nov.	*P. rhodesiae* sp. nov.	*P. aeruginosa*	*P. fluorescens* biovar I	*P. fluorescens* biovar II	*P. fluorescens* biovar III	*P. fluorescens* biovar IV	*P. fluorescens* biovar V	*P. chlororaphis*	*P. aureofaciens*	*P. putida* biovar A	*P. putida* biovar B
Pyocyanin production	−	−	+	−	−	−	−	−	−	−	−	−
Lipase production	−	−	+	D	−	D	D	D	D	D	D	D
Denitrification	+	−	+	−	+	+	+	−	+	+	−	−
Growth at 4°C	+	+	−	+	+	+	+	D	+	+	D	+
Growth at 41°C	−	−	+	−	−	−	−	−	−	−	−	−
Lecithinase production	−	+	−	+	D	+	+	D	+	D	−	−
Gelatin liquefaction	D	−	+	+	+	+	+	+	+	+	−	−
Assimilation of:												
D-Ribose, mannitol	+	+	+	+	+	D	+	D	+	+	D	D
D-Xylose	+	+	−	+	D	D	D	D	−	−	D	D
L-Arabinose	+	+	−	+	+	D	+	D	−	+	D	+
L-Rhamnose	−	−	−	−	D	D	−	D	−	−	−	−
Glucose, Gluconate	+	+	+	+	+	+	+	+	+	D	+	+
D-Mannose	+	+	−	+	+	+	+	D	+	+	D	D
D-Galactose	+	+	−	+	+	D	+	D	D	+	−	D
D-Fructose	+	+	+	+	D	+	+	+	D	D	+	+
Sucrose	+	+	−	+	+	−	+	D	+	D	−	D
Trehalose	D	+	−	+	+	D	+	D	+	D	−	−
2-Ketogluconate	+	+	+	+	+	+	D	+	+	D	D	+
Mucate	D	+	−	+	+	D	+	+	+	+	D	+
Propionate	+	+	+	+	+	D	+	+	+	+	+	+
Butyrate	+	−	+	−	D	D	+	D	+	D	+	+
Isobutyrate	+	−	+	−	D	D	−	D	−	D	D	D
Valerate	+	+	+	D	D	D	−	D	+	+	+	+
Isovalerate	−	D	+	D	D	D	−	D	+	+	+	+
Caproate	+	+	+	+	D	+	+	+	D	+	+	+
Malonate	+	+	+	+	+	D	+	D	+	+	D	+
Adipate	D	−	+	−	−	D	−	−	−	−	−	D
Sebacate	−	−	+	−	−	D	−	−	−	−	−	D
Pimelate, suberate	−	−	D	−	−	D	−	−	−	−	−	−
Azelate	−	−	+	−	−	D	−	−	−	−	−	−
D-Malate	−	−	D	−	D	D	+	D	D	−	D	D
D-Tartrate	−	−	−	−	D	−	−	D	−	−	D	D
L-Tartrate	−	−	−	−	−	−	+	−	D	−	D	D
m-Tartrate	−	−	−	−	−	D	−	D	−	−	D	−
Glycolate	D	−	−	−	−	−	−	−	−	−	D	−
Glycerate	+	+	+	+	+	D	+	D	+	+	D	+
Aconitate	+	+	+	+	+	D	+	D	D	+	+	+
Erythritol	+	−	−	D	D	+	−	D	−	−	−	−
Sorbitol	+	+	−	+	+	D	+	D	−	−	−	D
Inositol	+	+	−	D	+	D	+	D	+	+	−	−
Adonitol	−	−	−	+	−	D	−	D	−	−	−	−
D-Mandelate	−	−	−	−	−	−	−	−	−	−	D	D
L-Mandelate	−	−	+	−	−	−	−	D	−	−	−	D
Benzoate	−	−	+	D	D	D	+	D	+	D	D	+
o-Hydroxybenzoate	−	−	−	−	−	−	−	−	D	−	D	D
m-Hydroxybenzoate	−	−	−	−	−	−	−	−	D	D	D	D
p-Hydroxybenzoate	+	+	+	+	+	D	+	D	+	+	+	+
Phenylacetate	−	−	−	−	−	D	−	D	D	+	D	+
α-Aminobutyrate	+	+	−	−	−	−	−	−	−	−	−	D
D-Tryptophan	−	−	−	−	−	−	−	−	−	−	−	D
Creatine	−	−	−	−	−	D	−	−	−	−	D	D
Glycine	−	−	D	−	−	D	−	D	−	−	D	+
D-Alanine	+	+	+	+	+	+	+	−	+	−	+	+
L-Serine	+	+	D	+	D	+	+	D	D	+	D	D
L-Leucine	D	+	+	+	D	+	+	+	+	+	+	+
L-Isoleucine, L-valine	+	+	D	+	+	+	+	+	+	+	+	+
L-Lysine	−	−	+	+	D	D	+	D	D	D	+	D
L-Ornithine	D	−	+	+	D	D	D	D	D	+	+	+
L-Citrulline	D	−	D	D	D	D	−	D	D	D	D	D
L-Histidine	D	+	+	+	D	+	+	D	+	+	+	+
L-Phenylalanine	+	+	D	D	D	D	+	D	D	+	+	+
L-Tryptophan	+	−	D	+	D	D	−	D	+	+	−	+
L-Kynurenine	−	−	+	D	D	D	−	D	+	+	−	+
Ethanolamine	+	+	D	+	D	D	+	D	D	+	D	D
Benzylamine	−	−	−	−	−	D	−	D	−	−	D	+
Histamine	−	−	+	D	−	D	−	D	D	D	D	+
Tryptamine	−	−	−	−	D	D	−	−	−	−	D	+
Butylamine	−	−	−	−	−	−	−	D	−	D	+	+
Amylamine	−	−	−	−	D	D	−	D	−	+	D	+
Sarcosine	+	+	D	+	D	D	+	D	D	+	+	+
Acetamide	−	−	+	−	−	−	−	−	−	−	D	D
Trigonelline	+	−	−	D	D	D	−	D	−	−	D	+

[a] Symbols: +, 90% or more of strains are positive; −, 90% or more of strains are negative; D, 11 to 89% of strains are positive.

RESULTS

Biochemical, physiological, and flagellation characteristics. The phenotypic data of strains belonging to cluster Ib have been described previously (17) and have been compared with those of *P. rhodesiae* (8) and other nonpathogenic members of section I of pseudomonads (38) (see Table 3). A single polar flagellum on strain CFML 92-134[T] was observed by transmission electron microscopy (Fig. 1).

DNA-DNA hybridization. The DNA-DNA hybridization values between strain CFML 92-134[T] and seven other strains of cluster Ib are given in Table 1. The relative binding ratio within this cluster was greater than 80% at 60°C (Table 1). The ΔT_m values were less than 1°C. Results of DNA-DNA hybridizations with members of two other phenotypic clusters previously described by Elomari et al. (17) are also given in Table 1. The first cluster (IIa), which contained 14 wild strains isolated from natural mineral waters, could be divided into two subclusters, IIa1 and IIa2, on genomic grounds. The subcluster IIa1 formed a homogeneous genomic group for which *P. rhodesiae* sp. nov. (8) was proposed. The second phenotypic cluster, V, contained 13 mineral water strains and three culture collection strains (ATCC 17559, ATCC 17571, and ATCC 17400) of *P. fluorescens* biovar III. The levels of DNA hybridization of the representative strain of cluster Ib CFML 92-134[T] and all strains of cluster IIa and nine representative strains of cluster V varied between 31 and 56% for subcluster IIa1 (Table 1), 20 to 42% for subcluster IIa2, and 33 to 41% with cluster V (Tables 1 and 2). Hybridization values between strain CFML 92-134[T] and a great number of strains belonging or related to species of section I of the genus *Pseudomonas* (32), the related species *P. lundensis*, *P. fragi*, *P. savastanoi*, and fluorescent members of *Pseudomonas* section V (32) were 47% or lower, with ΔT_ms between 8 and 20°C (Table 2). The highest hybridization values were obtained with the different biovars of *P. fluorescens* and with representative strains of *P. marginalis*, *P. chlororaphis*, and *P. aureofaciens*.

TABLE 4. Variable characteristics among eight strains of *P. veronii*

Characteristic	Reaction of most strains[a]	Reaction of type strain
Conventional tests		
Tributyrine esterase, malonate	− (2)	−
Gelatinase	− (1)	+
Mucate	+ (3)	−
Urea	− (2)	+
Elastase	+ (2)	+
Utilization of:		
N-Acetyl-glucosamine	+ (2)	−
Trehalose, L-leucine, glutarate, L-histidine	+ (1)	+
Adipate	C (4)	+
Glycolate	+ (3)	+
Levulinate	+ (2)	+
2-Ketoglutarate	− (3)	−
L-Serine	− (2)	+
L-Tyrosine	+ (1)	−
L-Ornithine, L-citrulline	− (2)	−
DL-2-Amino-benzoate	− (1)	−
Enzymatic tests		
Esterase C_{12}, L-seryl-tyrosine arylamidase, glycyl-L-tryptophan arylamidase	+ (3)	+
L-Tyrosine arylamidase	− (3)	−
L-Pyrrolidone arylamidase	C (4)	−
L-Hydroxyproline arylamidase, γ-glutamyl transferase, L-arginine arylamidase, L-lysyl-L-lysine arylamidase, α-L-aspartyl-L-arginine arylamidase, L-phenylalanyl-L-prolyl-L-alanine arylamidase	+ (1)	+
L-Histidine arylamidase, N-acetyl-glycyl-L-lysine arylamidase, N-CBZ[b]-arginyl-4-methoxy arylamidase, α-L-glutamate arylamidase, L-isoleucine arylamidase	C (4)	+
L-Aspartate arylamidase, *S*-benzyl-cysteine arylamidase, L-alanyl-L-phenylalanyl-L-prolyl-L-alanine arylamidase	− (2)	−
Glycyl-proline arylamidase, L-tryptophane arylamidase, glycyl-L-arginine arylamidase, L-prolyl-L-arginine arylamidase	+ (2)	+
L-Threonine arylamidase	− (3)	+
α-L-Glutamyl-L-histidine arylamidase	− (1)	+
L-Histidyl-L-serine arylamidase	− (2)	+

[a] The number in parentheses is the number of strains deviating from the most common result. C, 50% of strains are positive.
[b] CBZ, carboxybenzoxy.

DNA base composition. The G+C contents of the eight strains studied (cluster Ib) were between 61 and 62 mol% (Table 1).

DISCUSSION

Forty-six strains isolated from mineral water and phenotypically identified as fluorescent pseudomonads were grouped in three phenotypic clusters named Ib, IIa, and V (17). The phenotypic cluster Ib is the most similar to cluster Ia, containing *P. fluorescens* biovar III and *P. marginalis*, emphasizing that cluster Ib is indeed a member of the authentic pseudomonads. This is confirmed by the hybridization results with up to 47% DNA-DNA hybridization with *P. fluorescens* biovar II strains (Table 2). Other subclusters of cluster I (17) contain members of *P. fluorescens* biovars I, II, and V. Moreover, the gene for the OprI lipoprotein (14) that is typical for members of pseudomonads of rRNA group I could also be amplified as a unique fragment in strains of group Ib. These results clearly show that group Ib indeed belongs to the genus *Pseudomonas* sensu stricto (12). In order to further unravel the finer taxonomic position of the group Ib within this genus, we performed DNA-DNA hybridizations within this cluster containing eight strains and with the type and representative strains of other related groups or species (Tables 1 and 2). The results obtained in this study demonstrate that cluster Ib constitutes a separate DNA hybridization group (80 to 100% hybridization) with only low levels of DNA hybridization with other species belonging to the saprophytic fluorescent pseudomonads (7 to 47%) and with possible related *Pseudomonas* species, including the newly proposed species *P. rhodesiae* (8). The latter species, created as part (IIa1) of the members of cluster IIa, also contains solely isolates from mineral waters. The DNA-DNA hybridizations obtained between strain CFML 92-134^T and all strains of cluster IIa were feeble (Table 1). The levels of DNA relatedness between the representative Ib strain CFML 92-134^T and six new isolates and *P. fluorescens* biovar III strains (ATCC 17559, ATCC 17571, and ATCC 17400) from cluster V were low (Tables 1 and 2). The ΔT_ms of hybrids formed between strain CFML 92-134^T and the seven other strains of cluster Ib were less than 1°C. The ΔT_ms obtained for hybrids between strain CFML 92-134^T and reference strains of other possible related species of the genus *Pseudomonas* were high (8 to 20°C). At present, the definition of a genomic species (45) includes the requirements that within a species, strains should have DNA hybridization of 70% or more, with a ΔT_m of 5°C or less, and that the results of other techniques (including phenotypical analysis) should decide if a genomic species deserves the status of a species. Various phenotypic characteristics, such as lecithinase production, growth at 4 or 41°C, and assimilation of D-xylose, erythritol, sorbitol, inositol, benzoate, L-kynurenine, trigonelline, isovalerate, L-arabinose, and isobutyrate (Table 3), were found to differentiate the Ib group members from *P. rhodesiae* (8) and from the other *Pseudomonas* species and groups. Details concerning D responses in Table 3 are given in Table 4. Consequently, we conclude from the results of this study and from previous work that group Ib deserves a separate species status, for which we propose the name *P. veronii.*

Description of *P. veronii* sp. nov. *P. veronii* (ve.ro'ni.i. M.L. masc. gen. n. *veronii*, of Véron, in honor of Prof. M. M. Véron, an eminent French microbiologist, for his contribution to taxonomy and medical microbiology).

The cells are gram negative; oxidase, catalase, and arginine dihydrolase positive; and motile by means of a single polar flagellum (Fig. 1). They produce a fluorescent pigment on King B medium. Growth occurs between 4 and 36°C but not at 41°C. The species denitrifies. Poly-B-hydroxybutyrate is not accumulated. Colonies on nutrient agar are smooth, circular, and nonpigmented. They are nonhemolytic on blood agar. The phenotypic characteristics have been given previously (17). All these strains grow on α-aminobutyrate, D-xylose, L-arabinose, D-mannose, D-galactose, sucrose, butyrate, isobutyrate, erythritol, sorbitol, inositol, D-alanine, L-tryptophan, and trigonelline as the sole source of carbon and energy, but none is able to utilize isovalerate, sebacate, azelate, L-mandelate, benzoate, L-kynurenine, histamine, or acetamide.

The G+C content of the DNA is 61 to 62 mol%. All strains have been isolated from natural mineral waters. No clinical significance is known; the type strain is CFML 92-134^T and has

been deposited at the Collection Institut Pasteur (CIP) as CIP 104663^T. The G+C content of the type strain is 61 mol%.

ACKNOWLEDGMENTS

We gratefully acknowledge M. Simonet for his helpful advice and critical review of the manuscript, and we also thank C. Dumoulin for her technical assistance.

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