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Pseudomonas monteilii sp. nov., Isolated from Clinical Specimens

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We propose the name *Pseudomonas monteilii* for a new species of gram-negative, rod-shaped, motile bacteria that were nonhemolytic on blood agar and were isolated from clinical sources. The 10 strains of *P. monteilii* were incapable of liquefing gelatin. They grew at 10°C but not at 41°C, produced fluorescent pigments, catalase, and cytochrome oxidase, and possessed the arginine dihydrolase system. They were capable of respiratory but not fermentative metabolism. They did not hydrolyze esculin or starch and were able to use benzylamine, α-aminobutyrate, D-ribose, L-arabinose, butyrate, valerate, isovalerate, isobutyrate, inositol, phenylacetate, D-alanine, and amylamine. They possessed L-phenylalanine arylamidase, L-lysine arylamidase, L-alanine arylamidase, γ-glutamyl-transferase, glycyl-phenylalanine arylamidase, L-tryptophan arylamidase, glycyl-L-alanine arylamidase, esterase C_4, esterase C_6, esterase C_8, esterase C_9, esterase C_{10}, and esterase C_{18}. DNA relatedness studies revealed that *P. monteilii* strains formed a homogeneous DNA hybridization group. A total of 57 strains representing previously described or partially characterized taxa belonging to the genus *Pseudomonas* were 6 to 54% related to *P. monteilii*. The highest hybridization values were obtained with strains belonging to or related to *Pseudomonas putida* biovar A. The average G+C content of the DNA was 60.5 ± 0.5 mol% for four of the *P. monteilii* strains studied. The type strain of *P. monteilii* is CFML 90-60 (= CIP 104883); it was isolated from bronchial aspirate and has a G+C content of 60 mol%. The clinical significance of these organisms is not known.

It is generally accepted today that the genus *Pseudomonas*, as described in *Bergey's Manual of Systematic Bacteriology* (32), was multigeneric and should not be maintained as a single genus (11–13, 33, 34, 53, 60, 61). The taxonomy of this genus is based mainly on the phenotypic characterization carried out by Stanier et al. (51) and the groups proposed by Palleroni et al. (38) on the basis of rRNA-DNA hybridization studies. Recent studies on pseudomonads have clarified their taxonomy (35) and have led to the creation of new genera, such as the genera *Stenotrophomonas* (36), *Comamonas* (54), *Acidovorax* (59), *Hydrogenophaga* (60), *Sphingomonas* (62), *Burkholderia* (64), *Ralstonia* (63), *Telluria* (4), and *Brevundimonas* (47). Therefore, the genus *Pseudomonas* sensu stricto should be restricted to *Pseudomonas* rRNA group I (12, 32), which includes both fluorescent and nonfluorescent species (*Pseudomonas stutzeri* [37, 56], *Pseudomonas mendocina*, *Pseudomonas alcaligenes* [42], and *Pseudomonas fragi* [30]).

Pseudomonas aeruginosa, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas chlororaphis* (5, 23, 39, 51), and the plant-pathogenic *Pseudomonas* species (*Pseudomonas syringae* and *Pseudomonas cichorii*) are the most important fluorescent species (34). Two other species, *Pseudomonas veronii* (16) and *Pseudomonas rhodesiae* (6), have been described recently for fluorescent *Pseudomonas* strains isolated from natural mineral waters (15). A common characteristic of all of the above organisms is the production of pigments that fluoresce under short-wavelength UV light (25). Taxonomically, the fluorescent pseudomonads are extremely complex. The plant-pathogenic fluorescent pseudomonads (17, 32) are characterized by a negative arginine dihydrolase reaction and represent a branch that is phylogenetically separate from the other fluorescent organisms in similarity group I of Palleroni (32).

P. aeruginosa, the type species of the genus *Pseudomonas*, is a typical opportunistic pathogen (3). Most strains of this species can be easily identified by a number of phenotypic characteristics (34). This is a homogeneous species on genotypic grounds (35).

P. fluorescens and *P. putida* were described a few years after *P. aeruginosa* was described. *P. putida* is particularly interesting. Recently, on the basis of a lot of research, workers have described a role for *P. putida* in aromatic hydrocarbon degradation (21, 24, 26, 27, 31, 40, 46, 48). Moreover, strains of *P. putida* are very common environmental contaminants, as mentioned by Hugh and Gilardi (22), and this organism is rarely pathogenic for humans, even though it has been found associated with urinary tract infections, septicemia, septic arthritis, osteomyelitis, wound infections, pelvic inflammatory disease, and various other diseases (33). In any event, although its virulence may be low, *P. putida* should be considered potentially pathogenic (58). There are numerous references to isolation from a variety of materials of clinical origin in the literature (2, 18, 19, 41, 43, 52, 57).

In 1994, we showed on the basis of a numerical analysis (14) of the phenotypic characteristics of 39 strains that there are four phenotypic subclusters (subclusters IIa to IId) among strains of clinical origin that belonging to or are related to *P. putida*. Subcluster IIb included collection strains and the type strain of *P. putida* biovar A, whereas subclusters IIa, IIc, and IId contained only wild strains. The purpose of this study was to determine the taxonomic position of subcluster IIc by using genotypic methods (DNA-DNA hybridization and the difference between the melting point of a heteroduplex and the melting point of a homoduplex [ΔT_m]). Here we formally describe a new species, *P. monteilii*, whose type strain is CFML 90-60 (= CIP 104883).

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MATERIALS AND METHODS

Bacterial strains. A total of 92 strains were studied. These organisms included 35 wild strains that were isolated from clinical specimens (Table 1) (14) and were received as *P. putida*. These strains were collected during two years (1987 and

TABLE 1. Strains used and levels of relative binding of DNAs from members of *Pseudomonas* section I and V species to [^{3}H]DNA from *P. monteilii* CFML 90-60^T

Taxon (as received) or phenotypic subcluster	Strain[a]	Labeled DNA from strain CFML 90-60^T		G+C content (mol%)
		Relative binding ratio (%)	ΔT_m (°C)	
Subcluster IIc	CFML 90-60^T	100	0.0	60
	CFML 90-56	99		61
	CFML 90-58	95		
	CFML 90-62	94		
	CFML 90-54	94		60.5
	CFML 90-61	93		60
	CFML 90-59	91		
	CFML 90-63	87	1.2	
	CFML 90-55	86	0.5	
	CFML 90-57	86	1.0	
Subcluster IIc	CFML 90-53	41	8.0	
	CFML 90-100	29		
Subcluster IIa	CFML 90-139	49	7.4	
	CFML 90-36	48	7.5	
	CFML 90-33	42	9.2	
	CFML 90-34	40	9	
Subcluster IIb	CFML 90-46	54	6.7	
	CFML 90-49	50	8.4	
	CFML 90-52	50	7.6	
P. putida biovar A	DSMZ 50208	48	7.9	
	CFML 90-48	45	8.8	
	CFML 90-39	41	9.8	
P. putida biovar A	ATCC 12633^T	40	9.2	
	CFML 90-42	40	7.6	
	CFML 90-47	37	10.5	
	CFML 90-51	36	7.9	
	CFML 90-40	35		
	CFML 90-50	35		
	CFML 90-43	30		
	CFML 90-38	29		
	CFML 90-37	24		
	CFML 90-44	20		
	CFML 90-45	21		
Subcluster IId	CFML 90-135	44	9.2	
	CFML 90-136	42	7.1	
	CFML 90-138	40	8.9	
	CFML 90-35	11		
P. putida biovar B	ATCC 17484	15		
P. putida biovar B	ATCC 17430	17		
P. putida biovar B	CCUG 1317	8		
P. aeruginosa	ATCC 10145^T	6		
P. aeruginosa	ATCC 27853	11		
P. aeruginosa	ATCC 15692	11		
P. fluorescens biovar I	ATCC 13525^T	16		
P. fluorescens biovar I	ATCC 17563	10		
P. fluorescens biovar I	ATCC 17397	26		
P. fluorescens biovar II	ATCC 17816	15		
P. fluorescens biovar II	DSMZ 50106	17		
P. fluorescens biovar II	ATCC 17815	20		
P. fluorescens biovar II	ATCC 17482	16		
P. fluorescens biovar III	ATCC 17559	13		
P. fluorescens biovar III	ATCC 17400	18		
P. fluorescens biovar IV	DSMZ 50415	19		
P. fluorescens biovar IV	ATCC 12983	15		
P. fluorescens biovar V	ATCC 14150	16		
P. fluorescens biovar V	ATCC 17518	17		
P. fluorescens biovar V	ATCC 15916	18		
P. fluorescens biovar V	ATCC 17386	33	14.3	
P. fluorescens biovar V	DSMZ 50148	18		
P. fluorescens biovar V	ATCC 17573	16		
P. marginalis	ATCC 10844^T	17		
P. marginalis	DSMZ 50275	20		
P. marginalis	DSMZ 50276	18		
P. chlororaphis	DSMZ 50083^T	23		
P. chlororaphis	ATCC 9447	25		
P. chlororaphis	ATCC 17414	18		
P. aureofaciens	CCEB 518^T	11		
P. aureofaciens	ATCC 17415	17		
P. lundensis	CCM 573^T	7		
P. lundensis	CCUG 18758	10		
P. syringae	ATCC 19310^T	11		
P. savastanoi	CFBP 1670^T	11		
P. savastanoi	CFBP 2088	12		
P. savastanoi	CFBP 1838	12		
P. viridiflava	ATCC 13223^T	11		
P. cichorii	DSMZ 50259^T	14		
P. agarici	ATCC 25941^T	13		
P. asplenii	ATCC 23835^T	13		
P. caricapapayae	NCPPB 1873^T	6		
P. tolaasii	NCPPB 2192^T	22		
P. tolaasii	NCPPB 1616	18		
P. stutzeri	ATCC 17588^T	9		
P. stutzeri	ATCC 17591	8		
P. stutzeri	ATCC 17587	8		
P. stutzeri	ATCC 17686	6		
P. mendocina	ATCC 25411^T	9		
P. mendocina	ATCC 25412	11		
P. alcaligenes	ATCC 14909^T	14		
P. pseudoalcaligenes	ATCC 17440^T	6		
P. pseudoalcaligenes	ATCC 12815	10		
P. fragi	ATCC 4973^T	13		
P. fragi	ATCC 27362	15		

[a] Abbreviations: CFML, Collection de la Faculté de Médecine de Lille, Lille, France; ATCC, American Type Culture Collection, Rockville, Md.; CCEB, Culture Collection of Entomogenous Bacteria, Institute of Entomology, Czechoslovakia Academy of Sciences, Prague, Czech Republic; CCM, Czechoslovak Collection of Microorganisms, J.E. Purkyne University, Brno, Czech Republic; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland, United Kingdom; NCPPB, National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory, Hatching Green, Harpenden, England, United Kingdom; PDDCC, Culture Collection of Plant Diseases Division, New Zealand Department of Scientific and Industrial Research, Auckland, New Zealand.

1989). A total of 57 type and collection strains were also included in this study for control purposes (Table 1). All of the bacteria were cultured routinely on Mueller-Hinton medium plates which were incubated at 30°C.

Conventional, assimilation, and enzymatic tests. Conventional, assimilation, and enzymatic tests were performed previously (14).

DNA hybridization experiments and thermal stability of duplexes. DNA extraction was performed as described previously (1, 28). The purity and quality of each DNA preparation were checked by determining the A_{260}/A_{280} and A_{260}/A_{230} ratios as described by Marmur and Doty (29). Native DNA was labeled in vitro with [^{3}H]cytosine by nick translation (20). The procedure used for the hybridization experiments (the S1 nuclease-trichloroacetic acid method) has been described previously (7, 20). The thermal stability of duplexes was determined by using the method of Crosa et al. (7). The divergence between DNAs was estimated by determining the ΔT_m value.

TABLE 2. Sources of *P. monteilii* strains

Strain[a]	Source	Sender
CFML 90-54	Placenta	Hansen Bruxelle University Hospital
CFML 90-55	Stool	Lille University Hospital
CFML 90-56	Bile	Lille University Hospital
CFML 90-57	Biological fluid	Freney Lyon University Hospital
CFML 90-58	Clinical isolate	Freney Lyon University Hospital
CFML 90-59	Clinical isolate	Lille University Hospital
CFML 90-60^T	Bronchial aspirate	Freney Lyon University Hospital
CFML 90-61	Bronchial aspirate	Lille University Hospital
CFML 90-62	Urine	Monteil Strasbourg University Hospital
CFML 90-63	Pleural fluid	Lille University Hospital

[a] CFML, Collection de la Faculté de Médecine de Lille, Lille, France.

G+C content determination. The G+C content was calculated from the thermal denaturation curve by using the equation of De Ley (8).

PCR amplification. PCR amplification was performed with a model 480 DNA thermal cycler (Perkin-Elmer Corp.) by using a PCR mixture (final volume, 100 μl) containing each of the deoxynucleoside triphosphates at a concentration of 200 μM, each of the primers (PS1 and PS2) (10) at a concentration of 1 mM, 1 μg of target DNA, and 2.5 U of *Taq* DNA polymerase. A total of 30 cycles of amplification were performed; each cycle consisted of template DNA denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, and primer extension at 72°C for 2 min. Before the DNA polymerase was added, each sample was denatured at 94°C for 5 min. A 10-μl portion of the amplified fragment preparation was loaded onto a 1% (wt/vol) agarose gel for electrophoresis.

The specificity of the amplified DNA was confirmed by Southern blot hybridization with an *OprI* DNA probe. This probe was obtained from the PCR products after amplification of the DNA of the *OprI* gene-positive reference strain (10, 44). The amplified fragment was desalted by low-melting-point agarose gel electrophoresis and purification by using a GeneClean kit (Amersham) and was labeled with horseradish peroxidase by using the ECL labeling system (Amersham). The methods used for Southern transfer and hybridization have been described previously (45); 100-bp DNA (Gibco BRL) was used as a molecular weight marker.

RESULTS

Conventional, assimilation, and enzymatic tests. The phenotypic results obtained for all subcluster IIc strains have been published previously (14). The results obtained for the 10 strains included in Table 2 are shown in Table 3 and were compared to the results obtained for pathogenic or saprophytic members of section I of Palleroni (32).

DNA relatedness. The results of DNA relatedness experiments obtained with labeled DNA of subcluster IIc strain CFML 90-60^T are presented in Table 1. The levels of hybridization between strain CFML 90-60^T and the 11 other isolates belonging to the same subcluster ranged from 29 to 100%. Ten strains of this subcluster were 86 to 100% related (Table 1). For the three lowest relatedness values (86, 86, and 87%), the ΔT_m values were 1, 0.5, and 1.2°C, respectively. The two other strains of this phenotypic subcluster, strains CFML 90-53 and CFML 90-100, had relative binding ratios of only 29 and 41%, respectively, with labeled DNA from strain CFML 90-60^T. Hybridization experiments were also performed with all of the strains of the three other phenotypic subclusters described previously (subclusters IIa, IIb and IId) (14). Phenotypic subcluster IIb contained 15 clinical isolates and two culture collection strains of *P. putida* biovar A (ATCC 12633^T and DSMZ 50208). Phenotypic subclusters IIa and IId each included four wild strains, all of which were isolated from clinical specimens. The levels of DNA relatedness between strain CFML 90-60^T and the strains of the other subclusters varied between 21 and 54% for subcluster IIb strains, between 40 and 49% for subcluster IIa strains, and between 11 and 44% for subcluster IId strains (Table 1). The ΔT_m values were 6.7 to 10.5°C. The levels of reassociation between the reference strain of subcluster IIc and cultures belonging to the different species of *Pseudomonas* section I of Palleroni (32), the possibly related species *Pseudomonas lundensis*, *P. fragi*, and *Pseudomonas savastanoi*, and fluorescent members of *Pseudomonas* section V of Palleroni (32) are also shown in Table 1. For all of these taxa (except *P. putida* biovar A) the levels of relatedness were in the range of 6 to 33%.

DNA base composition. The G+C contents of strains CFML 90-60^T, CFML 90-56, CFML 90-54, and CFML 90-61 were 60, 61, 60.5, and 60 mol%, respectively (Table 1), as determined by the thermal denaturation procedure.

PCR amplification. A fragment of the expected size (249 bp) was amplified by PCR for all 10 strains (Table 2) of subcluster IIc. Identical results were obtained after Southern blot hybridization with the *OprI* DNA probe from the reference strain (10, 44).

DISCUSSION

A total of 37 of the 39 strains isolated from clinical specimens and phenotypically identified as fluorescent pseudomonads were members of one phenotypic cluster, cluster II, which could be subdivided into four subclusters, subclusters IIa to IId (14). Phenotypically, subcluster IIc is most similar to subcluster IIb, which contains the type and collection strains of *P. putida* biovar A.

In order to further determine the taxonomic position of subcluster IIc within *Pseudomonas* sensu stricto (12), we performed DNA-DNA hybridization experiments with members of this subcluster (12 strains) and with the type strains and other representative strains of other species of this genus (Ta-

TABLE 3. Characteristics of 10 *P. monteilii* strains

Characteristic	Reaction of most strains	Reaction of type strain
Conventional tests		
Tyrosine	+ (4)[a]	+
Urea	b	+
Cetrimide	+ (1)	+
2,3,5-Triphenyltetrazolium chloride	+ (2)	+
Growth on phenylpyruvic acid or at 4°C	− (1)	−
Utilization of:		
Glycine, L-tyrosine	− (1)	−
L-Histidine, creatine, L-phenylalanine	+ (1)	+
L-Citrulline	− (3)	−
DL-Norvaline	+ (4)	+
L-Serine, histamine	+ (2)	+
L-Threonine	− (4)	−
Trigonelline	+ (3)	−
Malonate	+ (4)	−
D-Malate	b	−
Enzymatic tests		
L-Proline arylamidase, methionine arylamidase	+ (3)	+
L-Pyrrolidone arylamidase	− (1)	+
L-Arginine arylamidase, L-lysyl-L-alanine arylamidase, L-seryl-L-tyrosine arylamidase	− (1)	−
L-Glycyl-L-tryptophan arylamidase, esterase C_{12}	+ (2)	+
L-Seryl-L-methionine arylamidase	b	−

[a] +, positive; −, negative; b, 50% of the strains are positive. The numbers in parentheses are the numbers of strains that deviate from the most common result.

ble 1). The results obtained show that 10 subcluster IIc strains form a homogeneous group with levels of hybridization of 86 to 100% (Table 1). The ΔT_m values were less than 1.2°C, indicating that these organisms are sufficiently closely related to warrant recognition as members of a single species since at present the definition of a genomic species (49) states that the strains of a species, should have DNA hybridization values of 70% or more and ΔT_m values of 5°C or less (both values must be considered) and that the results of other techniques should decide if a genospecies deserves species status. The two other phenotypic subcluster IIc strains, CFML 90-53 and CFML 90-100, were expelled from the group despite the fact that they are phenotypically indistinguishable from the 10 other strains of this subcluster. These two strains may represent one or two additional genospecies for which supplementary studies will be needed to determine their taxonomic status and their relationships to the genus *Pseudomonas*.

The levels of hybridization between strain CFML 90-60^T and members of other species belonging to the genus *Pseudomonas* were low. The highest hybridization values were obtained with biovar A strains of *P. putida* (level of hybridization with collection strain DSMZ 50208, 48%), emphasizing that our group of 10 strains belongs to the genus *Pseudomonas* sensu stricto (13). These 10 strains share 20 characteristics with *P. aeruginosa*, *P. fluorescens* (all biovars), *P. chlororaphis*, and *P. putida* (biovars A and B), and, like these species, they were unable to use 10 other substrates as sole carbon and energy sources. In addition, the PCR for the gene for *OprI* lipoprotein (10), which could be amplified only in members of the authentic genus *Pseudomonas*, was positive when the 10 strains of subcluster IIc were tested.

The average DNA G+C content was 60.5 ± 0.5 mol% for the four strains studied. This value is typical of the genus *Pseudomonas* sensu stricto. Determination of the G+C content is one of the classical genotypic methods and is considered part of the standard description of a bacterial taxon. Generally, the range observed is not more than 3% within a well-defined species and not more that 10% within a well-defined genus (9, 50, 55).

On the basis of the results described above (DNA-DNA hybridization, ΔT_m, and G+C content data), it appeared that the 10 strains of subcluster IIc (Table 2) represented a new *Pseudomonas* species, for which we propose the name *Pseudomonas monteilii*. The sources of these strains are given in Table 2.

Description of *Pseudomonas monteilii* sp. nov. *Pseudomonas monteilii* (mon.tei'li.i M. L. masc. gen. n. *monteilii*, of Monteil, in honor of Henri Monteil, a French microbiologist, for his contribution to medical microbiology).

Biochemical and physiological characteristics. The 10 *P. monteilii* strains are gram negative, motile, asporogenous, and rod shaped. Colonies on nutrient agar are circular and nonpigmented. They are nonhemolytic on blood agar. The cells are lipase, elastase, lecithinase, and tetrathionate reductase negative. Arginine dihyrolase, catalase, and cytochrome oxidase are produced. The temperature range for growth is 10 to 36°C, and optimal growth occurs at 30°C. Growth occurs in the presence of 3% NaCl but not in the presence of 5 or 7% NaCl. No strain reduces nitrate to nitrite or to gas; the strains are not able to form levan from sucrose but they are able to use citrate and arginine. Tributyrin and fibrinolysis tests are negative.

Poly-β-hydroxybutyrate is not accumulated as a carbon reserve material. Phenazine pigments are not produced on King A medium (25). Strains are unable to grow at 41°C. Indole, coagulase, and β-xylosidase are not produced. Gelatin is not liquefied. Esculin and starch are not hydrolyzed. Phenylalanine is not deaminated, and lysine and ornithine are not decarboxylated. The Voges-Proskauer reaction and the *o*-nitrophenyl-β--galactopyranoside test are negative. There is no action against DNA or RNA. The strains are chondroitin negative.

Assimilation of carbon compounds. All strains utilize the following substrates as carbon and energy sources: glycerol, D-ribose, inositol, D-glucose, D-fructose, L-glutamate, L-arabinose, 2-ketogluconate, DL-glycerate, DL-3-hydroxybutyrate, gluconate, isobutyrate, L-proline, D-alanine, L-alanine, L-leucine, L-isoleucine, L-norleucine, L-valine, L-aspartate, L-ornithine, L-lysine, L-arginine, betaine, β-alanine, sarcosine, DL-4-aminobutyrate, DL-5-aminovalerate, butylamine, amylamine, ethanolamine, benzylamine, diaminobutane, spermine, aconitate, phenylacetate, succinate, fumarate, L-malate, acetate, propionate, butyrate, *n*-valerate, isovalerate, *n*-caproate, heptanoate, caprylate, caprate, glutarate, DL-lactate, puryvate, citrate, benzoate, and *p*-hydroxybenzoate.

No strain uses the following substrates as sole carbon and energy sources: L-xylose, α-methyl-D-mannoside, salicin, lactose, D-melibiose, inulin, D-tagatose, D-fucose, erythritol, D-arabinose, D-xylose, adonitol, β-methylxyloside, D-galactose, D-mannose, L-sorbose, L-rhamnose, dulcitol, sorbitol, α-methyl-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin, D-cellobiose, maltose, sucrose, trehalose, D-melezitose, D-raffinose, starch, glucogen, xylitol, β-gentobiose, D-turanose, D-lyxose, L-fucose, D-arabitol, 5-ketogluconate, L-cysteine, D-tryptophan, DL-2-aminobutyrate, L-methionine, L-tryptophan, DL-kynurenine, DL-3-aminobutyrate, DL-2-aminobenzoate, DL-3-aminobenzoate, DL-4-aminobenzoate, ethylamine, acetamide, tryptamine, glucosamine, levulinate, iso-phthalate, oxalate, maleate, adipate, pimelate, suberate, azelate, sebacate, D-tartrate, L-tartrate, *meso*-tartrate, citrate, mesaconate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, D-mandelate, L-mandelate, phthalate, terephthalate, glycerate, oxalate, L-arabitol, mannitol, glycolate, citraconate, itaconate, and mucate.

Enzymatic tests. All strains possess the following enzyme activities: L-phenylalanine arylamidase, L-lysine arylamidase, L-alanine arylamidase, γ-glutamyl-transferase, glycyl-phenylalanine arylamidase, L-tryptophan arylamidase, glycyl-L-alanine arylamidase, esterase C_4, esterase C_6, esterase C_8, esterase C_9, esterase C_{10}, and esterase C_{18}.

No strains possess the following enzyme activities: L-histidyl-L-phenylalanine arylamidase, glycyl-glycine arylamidase, L-hydroxyproline arylamidase, L-histidine arylamidase, glycine arylamidase, L-aspartate arylamidase, glycyl-proline arylamidase, leucyl-glycine arylamidase, L-glutamine arylamidase, α-L-glutamate arylamidase, L-isoleucine arylamidase, L-ornithine arylamidase, L-serine arylamidase, L-threonine arylamidase, *N*-carboxybenzoyl-glycyl-glycyl-arginine arylamidase, β-alanine arylamidase, L-alanyl-L-arginine arylamidase, L-alanyl-L-phenylalanyl-L-proline arylamidase, L-alanyl-L-phenylalanyl-L-prolyl-L-alanine arylamidase, L-arginyl-L-arginine arylamidase, α-L-aspartyl-L-alanine arylamidase, α-L-aspartyl-L-arginine arylamidase, α-L-glutamyl-L-histidine arylamidase, glycyl-L-arginine arylamidase, L-histidyl-L-leucyl-L-histidine arylamidase, L-histidyl-L-serine arylamidase, L-lysyl-L-lysine arylamidase, L-phenylalanyl-L-arginine arylamidase, L-phenylalanyl-L-proline arylamidase, L-phenylalanyl-L-prolyl-L-alanine arylamidase, L-seryl-L-methionine arylamidase, L-valyl-L-tyrosyl-L-serine arylamidase, L-lysyl-L-serine-4-methoxy-arylamidase, α-D-glucosidase, β-D-glucosidase, α-maltosidase, *N*-carboxybenzoyl-arginine-4-methoxy arylamidase, *N*-benzoyl-leucine arylamidase, *S*-benzoyl-cysteine arylamidase, α-L-glutamyl-α-L-glutamic arylamidase, L-leucyl-L-leucyl-L-valyl-L-tyrosyl-L-serine arylamidase, *N*-benzoyl-L-alanine-4-methoxy arylamidase, *N*-acetyl-glycyl-L-lysine arylamidase, α-D-galactosidase, β-D-galactosidase, phospho-β-D-galactosidase, α-L-ar-

TABLE 4. Differentiation of *P. monteilii* from nonphytopathogenic fluorescent members of section I of the genus *Pseudomonas*

Characteristic	*P. monteilii*	*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	−[a]	+	−	−	−	−	−	−	−	−	−
Lipase	−	+	d	−	d	d	d	d	d	d	d
Denitrification	−	+	−	+	+	+	−	+	+	−	−
Growth at 4°C	−	−	+	+	+	+	d	+	+	d	+
Growth at 41°C	−	+	−	−	−	−	−	−	−	−	−
Lecithinase	−	−	+	d	+	+	d	+	d	−	−
Gelatin liquefaction	−	+	+	+	+	+	+	+	+	−	−
Assimilation of:											
D-Ribose	+	+	+	+	d	+	d	+	+	d	d
D-Xylose	−	−	+	d	d	d	d	−	−	d	d
L-Arabinose	+	−	+	+	d	+	d	−	+	d	+
L-Rhamnose	−	−	−	d	d	−	d	−	−	−	−
Glucose	+	+	+	+	+	+	+	+	d	+	+
D-Mannose	−	−	+	+	+	+	d	+	+	d	d
Mannitol	−	+	+	+	d	+	d	+	+	d	d
D-Galactose	−	−	+	+	d	+	d	d	+	−	d
D-Fructose	+	+	+	d	+	+	+	d	d	+	+
Sucrose	−	−	+	+	−	+	d	+	d	−	d
Trehalose	−	−	+	+	d	+	d	+	d	−	−
Gluconate	+	+	+	+	+	+	+	+	d	+	+
2-Ketogluconate	+	+	+	+	+	d	+	+	d	d	+
Mucate	−	−	+	+	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	+	+	+	+
Caproate	+	+	+	d	+	+	+	d	+	+	+
Malonate	d	+	+	+	d	+	d	+	+	d	+
Adipate	−	+	−	−	d	−	−	−	−	−	d
Sebacate	−	+	−	−	d	−	−	−	−	−	d
Pimelate, suberate	−	d	−	−	d	−	−	−	−	−	−
Azelate	−	+	−	−	d	−	−	−	−	−	−
D-Malate	d	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	−
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	d
L-Leucine	+	+	+	d	+	+	+	+	+	+	+
L-Isoleucine, L-valine	+	d	+	+	+	+	+	+	+	+	+
L-Lysine	+	+	+	d	d	+	d	d	d	+	d
L-Ornithine	+	+	+	d	d	d	d	d	+	+	+
L-Citrulline	d	d	d	d	d	−	d	d	d	d	d
L-Histidine	+	+	+	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	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	−	−	d	+

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

abinosidase, β-D-galacturonohydrolase, β-D-glucuronidase, β-maltosidase, *N*-acetyl-α-D-glucosaminidase, β-D-fucosidase, β-L-fucosidase, β-D-lactosidase, α-D-mannosidase, β-D-mannosidase, α-D-xylosidase, β-D-xylosidase, *N*-acetyl-β-D-glucosaminidase, β-L-fucosidase, and esterase C_{16}.

Variable characteristics of 10 *P. monteilii* strains are shown in Table 3. All of the reactions of the type strain are the same as the reactions of the majority of the strains of *P. monteilii* except the reactions in the following three tests: assimilation of trigonelline and malonate and presence of L-pyrrolidone arylamidase. The *P. monteilii* strains are phenotypically and genotypically homogeneous and can be differentiated from related nonphytopathogenic fluorescent members of section I of the genus *Pseudomonas* (32) by several phenotypic features (Table 4). Differentiation of *P. monteilii* and *P. aeruginosa* is based on pyocyanin production, lipase activity, denitrification, growth at 41°C, and assimilation of L-arabinose, mannitol, adipate, sebecate, azelate, inositol, L-mandelate, phenylacetate, α-aminobutyrate, L-kynurenine, benzylamine, butylamine, amylamine, and acetamide. *P. monteilii* and *P. fluorescens* clearly differ phenotypically since all strains of *P. fluorescens* liquefy gelatin and are able to grow at 4°C, whereas strains of *P. monteilii* are unable to grow at this temperature and are unable to liquefy gelatin. Moreover, most strains of *P. fluorescens* utilize D-mannose, mannitol, D-galactose, and mucate, whereas all strains of *P. monteilii* are unable to utilize these compounds. Conversely, all strains of *P. monteilii* are able to grow on α-aminobutyrate, creatine (except one strain), and butylamine, whereas most strains of *P. fluorescens* fail to do so. Phenotypically, *P. monteilii* is most similar to *P. putida*, but these two species can be differentiated by assimilation of inositol, L-tartrate, D-tartrate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, tryptamine, D-mandelate, acetamide, D-xylose, mannitol, and mucate and by the presence of glycyl-glycine arylamidase, L-glutamine arylamidase, and L-arginine arylamidase (Table 4) (14).

Strains of *P. monteilii* were isolated from clinical specimens (Table 2). Type strain CFML 90-60 has been deposited in the Collection Institut Pasteur) as CIP 104883; it was isolated in Lyon, France, from a bronchial aspirate, and its G+C content is 60 mol%.

Clinical significance. The clinical significance of *P. monteilii* is not known. Future isolates of this new organism should be investigated to determine their role in nosocomial infections. At present, our hypothesis is that *P. monteilii* is a rare opportunistic pathogen or colonizer.

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