***Pseudomonas gessardii* sp. nov. and *Pseudomonas migulae* sp. nov., two new species isolated from natural mineral waters**

Sophie Verhille,¹ Nader Baïda,¹ Fouad Dabboussi,¹ Monzer Hamze,² Daniel Izard¹ and Henri Leclerc¹

Author for correspondence: Henri Leclerc. Tel: +33 3 20 52 94 28. Fax: +33 3 20 52 93 61.
e-mail: leclerc@univ-lille2.fr

¹ Service de Bactériologie-Hygiène, Faculté de Médecine Henri Warembourg (pôle recherche), 1 place de Verdun, 59045 Lille Cedex, France

² Faculté de Santé Publique, Université Libanaise, Tripoli, Lebanon and CNRS Liban, Beirut, Lebanon

Twenty-five non-identified fluorescent *Pseudomonas* strains isolated from natural mineral waters were previously clustered into three phenotypic subclusters, XIIIb, XVa and XVC. These strains were characterized genotypically in the present study. DNA–DNA hybridization results and DNA base composition analysis revealed that these strains were members of two new species, for which the names *Pseudomonas gessardii* sp. nov. (type strain CIP 105469^T) and *Pseudomonas migulae* sp. nov. (type strain CIP 105470^T) are proposed. *P. gessardii* included 13 strains from phenotypic subclusters XVa and XVC. *P. migulae* included 10 strains from phenotypic subcluster XIIIb. The levels of DNA–DNA relatedness ranged from 71 to 100% for *P. gessardii* and from 74 to 100% for *P. migulae*. The G+C content of the DNA of each type strain was 58 mol%. DNA similarity levels, measured with 67 reference strains of *Pseudomonas* species, were below 55%, with ΔT_m values of 13 °C or more. The two new species presented basic morphological characteristics common to all pseudomonads. Various phenotypic features were found to differentiate them: *P. gessardii* strains utilized L-arabitol, myo-inositol, adonitol, xylitol and meso-erythritol as carbon sources, whereas *P. migulae* strains assimilated L-arabinose, D-xylose, D-saccharate, meso-tartrate, tricarballylate, D-glucuronate, D-galacturonate, phenylacetate and histamine. The complete 16S rRNA sequences of each type strain were determined and compared with those of the type strains of *Pseudomonas* species. Finally, a phylogenetic tree was inferred from sequence analysis and demonstrated that the two new species fell into the '*Pseudomonas fluorescens* intrageneric cluster'. To date, their clinical significance is unknown.

Keywords: DNA–DNA hybridization, 16S rDNA, *Pseudomonas gessardii*, *Pseudomonas migulae*, mineral waters

INTRODUCTION

The genus *Pseudomonas* is one of the most complex genera of Gram-negative bacteria. This is why it was chosen by the European 'High-Resolution Automated Microbial Identification' (HRAMI) project as a subject for the evaluation of various taxonomic methods (Brosch *et al.*, 1996). Since its creation by Migula (1894), the major obstacle to clear-cut differentiation

of *Pseudomonas* from other groups of aerobic, polar-flagellated, Gram-negative bacteria has been its heterogeneity. The solution has been to use a variety of taxonomic criteria. Stanier *et al.* (1966) improved and clarified the situation by using a classification based on physiological and biochemical features, but the major breakthrough for the taxonomy of the genus *Pseudomonas* has resulted from the increasing use of genetic techniques. DNA–rRNA hybridization experiments carried out by Palleroni *et al.* (1973) clustered *Pseudomonas* into five rRNA groups. De Ley (1992) unravelled the taxonomic position of these five rRNA clusters within the general framework of the Gram-

The EMBL accession numbers for the 16S rRNA gene sequences of *P. gessardii* CIP 105469^T and *P. migulae* CIP 105470^T are AF074384 and AF074383.

negative bacteria. This has resulted in the drastic restriction of authentic *Pseudomonas* species to rRNA group I organisms, because it contains the type species, *Pseudomonas aeruginosa*. Rearrangement of the genus *Pseudomonas* has entailed the creation of several new genera, while other species have been reassigned to pre-existing genera, such as *Chryseomonas* and *Flavimonas* (Holmes *et al.*, 1987), *Burkholderia* (Yabuuchi *et al.*, 1992), *Ralstonia* (Yabuuchi *et al.*, 1995), *Comamonas* (De Vos *et al.*, 1985), *Acidovorax* (Willems *et al.*, 1990), *Hydrogenophaga* (Willems *et al.*, 1989), *Telluria* (Bowman *et al.*, 1993), *Stenotrophomonas* (Palleroni & Bradbury, 1993), *Brevundimonas* (Segers *et al.*, 1994), *Aminobacter* (Urakami *et al.*, 1992), *Oligotropha* (Meyer *et al.*, 1993), *Zavarzinia* (Meyer *et al.*, 1993), *Sphingomonas* (Yabuuchi *et al.*, 1990), *Herbaspirillum* (Baldani *et al.*, 1996) and *Devosia* (Nakagawa *et al.*, 1996). Nowadays, taxonomy is more and more defined as a system 'that should reflect as closely as possible natural relationships between bacteria, by using chronometer molecules such as RNA 16S' (Vandamme *et al.*, 1996). The natural intrageneric relationships of the genus *Pseudomonas sensu stricto* have been described by Moore *et al.* (1996), who delineated two major intrageneric clusters: '*Pseudomonas aeruginosa* intrageneric cluster' and '*Pseudomonas fluorescens* intrageneric cluster'. At the species level, the genus *Pseudomonas sensu stricto* includes at least 30 validly described species (Kerstens *et al.*, 1996). *Pseudomonads* are often metabolically versatile and are able to colonize low-nutrient-concentration ecosystems such as mineral waters (Oger *et al.*, 1987; van der Kooij, 1990). Natural mineral waters are characterized by their bacterial flora and their chemical and physical composition (Schwaller & Schmidt-Lorenz, 1981). However, a few studies have already demonstrated that identification at the species level may be a difficult task and sometimes requires further taxonomic study, including numerical analysis and genomic studies (Coroler *et al.*, 1996; Elomari *et al.*, 1996).

Twenty-five strains that could not be identified at the species level were previously clustered into three phenotypic subclusters (Verhille *et al.*, 1997): sub-cluster XIIIb (including strain CFML 95-321^T), sub-cluster XVa (including strain CFML 95-251^T) and sub-cluster XVc.

In this paper, we have characterized these strains genotypically to unravel the finer taxonomic position of these groups within the genus *Pseudomonas*. According to the outcome of the genomic study (DNA-DNA hybridization, ΔT_m and DNA G+C content), we propose the description of two new species, *Pseudomonas gessardii* sp. nov. (type strain CIP 105469^T = CFML 95-251^T) and *Pseudomonas migulae* sp. nov. (type strain CIP 105470^T = CFML 95-321^T).

METHODS

Bacterial strains. A total of 132 strains belonging to the genus *Pseudomonas* were investigated in this work. Sixty-five of

them were isolated from mineral waters and were previously allocated into the following ten phenotypic clusters or subclusters: IX, X, XI, XIIe, XIIIa, XIIIb, XIIIc, XIId, XVa and XVc (Verhille *et al.*, 1997). The other 67 strains were type or collection strains of authentic *Pseudomonas* species included as controls. Details concerning these strains are given in Tables 2-4.

Flagellation study. Negative staining was done with fixed cells of the type strains of *P. gessardii* and *P. migulae* according to the method described by Hoeniger (1965).

DNA extraction and purification. Extraction and purification of DNA were performed by the method of Beji *et al.* (1987).

DNA labelling and DNA-DNA hybridization. Native DNAs were labelled *in vitro* by nick translation with tritium-labelled nucleotides (Amersham). The S1 nuclease-trichloroacetic acid method used for hybridizations has been described previously (Grimont *et al.*, 1980). The re-association temperature was 60 °C. DNA-DNA hybridization experiments were carried out by using labelled DNAs from the type strains of *P. gessardii* (phenotypic subcluster XVa) and *P. migulae* (phenotypic subcluster XIIIb). Each measurement was repeated at least twice and means are shown.

Thermal stability of reassociated DNAs. The temperature at which 50% of reassociated DNA became hydrolysable by S1 nuclease (T_m) was determined by the method of Crosa *et al.* (1973). The ΔT_m was obtained as the difference between the T_m of the heteroduplex and the T_m of homoduplex.

DNA base composition. G+C contents were determined from the denaturation curve using the equation of De Ley (1970).

PCR amplification of 16S rDNA. Amplification of gene fragments encoding 16S rRNA was performed by using the following universal 16S rRNA primers described previously by Edwards *et al.* (1989), PA (5'-AGAGTTTGATCCTGGCTCAG-3') and PH (5'-AAGGAGGTGATCGAGCCGCA-3'), resulting in two overlapping DNA fragments that covered the 16S rRNA gene. One microgram of DNA was subjected to PCR in a total volume of 100 µl containing 10 µl 10 × buffer [50 mM KCl, 10 mM Tris/HCl, 15 mM MgCl₂, 10% (v/v) glycerol], 0.5 µl dNTP cocktail (1.25 mM each), 0.5 µl each primer (1 µM); 1.5 µl MgCl₂ (75 mM) and sterile distilled water to 100 µl. At least 0.5 µl (2.5 U) *Taq* DNA polymerase was added. Each 100 µl mixture was overlaid with three drops of mineral oil and subjected to thermal cycle amplification. A DNA thermal cycler 480 was used (Perkin-Elmer-Cetus) and programmed as follows: (i) an initial temperature of 94 °C for 5 min to melt the double-stranded DNA, (ii) 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C, and (iii) 10 min at 72 °C to allow extension of any incomplete products. The reaction mixture was held at 4 °C until it was used. Finally, 2 µl of the amplified fragment was loaded onto a 1.5% agarose gel for electrophoresis to check that only one band of the expected size was obtained.

The 16S rRNA gene sequences of *P. gessardii* CIP 105469^T and *P. migulae* CIP 105470^T were determined directly from PCR products in both the forward and reverse directions. An ABI 377 automated sequencer was used (Perkin-Elmer).

Analysis of sequence data. The two sequences generated were aligned with the 16S rDNA sequences of type strains of

Table 1. Type strains included in the phylogenetic tree

Culture collections are abbreviated as: IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; ATCC, American Type Culture Collection, Manassas, VA, USA; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; CIP, Collection de l'Institut Pasteur, Paris, France; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; NCPPB, National Collection of Plant-pathogenic Bacteria, Harpenden, UK; LMG, Laboratorium voor Microbiologie, Rijksuniversiteit Gent, Gent, Belgium.

Species	Strain	EMBL accession no.
<i>P. gessardii</i>	CIP 105469 ^T	AF074384
<i>P. migulae</i>	CIP 105470 ^T	AF074383
<i>P. aeruginosa</i>	DSMZ 50071 ^T	X06684
<i>P. agarici</i>	LMG 2112 ^T	Z76652
<i>P. alcaligenes</i>	LMG 1224 ^T	Z76653
<i>P. amygdali</i>	LMG 2123 ^T	Z76654
<i>P. asplenii</i>	LMG 2137 ^T	Z76655
<i>P. balearica</i>	DSMZ 6083 ^T	U26418
<i>P. caricapapayae</i>	ATCC 33615 ^T	D84010
<i>P. chlororaphis</i>	IAM 12354 ^T	D84011
<i>P. cichorii</i>	LMG 2162 ^T	Z76658
<i>P. citronellolis</i>	DSMZ 50332 ^T	Z76659
' <i>P. coronafaciens</i> '	LMG 13190 ^T	Z76660
<i>P. corrugata</i>	ATCC 29736 ^T	D84012
<i>P. ficuserectae</i>	LMG 5694 ^T	Z76661
<i>P. flavescens</i>	NCPPB 3063 ^T	U01916
<i>P. fluorescens</i> biovar I	DSMZ 50090 ^T	Z76662
<i>P. libanensis</i>	CIP 105460 ^T	AF057645
<i>P. marginalis</i> pv. <i>marginalis</i>	LMG 2210 ^T	Z76663
<i>P. mendocina</i>	LMG 1223 ^T	Z76664
<i>P. monteilii</i>	CIP 104883 ^T	AF064458
<i>P. mucidolens</i>	IAM 12406 ^T	D84017
<i>P. oleovorans</i>	DSMZ 1045 ^T	Z76665
<i>P. pseudoalcaligenes</i>	LMG 1225 ^T	Z76666
<i>P. putida</i>	DSMZ 291 ^T	Z76667
<i>P. resinovorans</i>	LMG 2274 ^T	Z76668
<i>P. rhodesiae</i>	CIP 104664 ^T	AF064459
<i>P. stutzeri</i>	CCUG 11256 ^T	U26262
<i>P. synxantha</i>	IAM 12356 ^T	D84025
<i>P. syringae</i>	LMG 12471 ^T	Z76669
<i>P. taetrolens</i>	IAM 1653 ^T	D84027
<i>P. tolaasii</i>	LMG 2342 ^T	Z76670
<i>P. veronii</i>	CIP 104663 ^T	AF064460
<i>P. viridiflava</i>	LMG 2352 ^T	Z76671

Pseudomonas species listed by Kersters *et al.* (1996) that were available in GenBank. These strains and their accession numbers are listed in Table 1. The multiple alignment was performed by using the CLUSTAL X program (a more up-to-date version of CLUSTAL W; Thompson *et al.*, 1994). A distance tree was constructed by the neighbour-joining method, correcting for multiple substitutions and excluding positions with gaps (Saitou & Nei, 1987). The tree was based

on a complete alignment of 16S rRNA sequences from 34 strains. These strains have been grouped according to the 16S rRNA sequence data of Kersters *et al.* (1996) (various *Pseudomonas* type strains), Coroler *et al.* (1996) (*Pseudomonas rhodesiae*), Elomari *et al.* (1996) (*Pseudomonas veronii*), Elomari *et al.* (1997) (*Pseudomonas monteilii*), and Dabboussi *et al.* (1999) (*Pseudomonas libanensis* CIP 105460^T). The tree topology was tested by a bootstrap analysis of 1000 resamplings (Felsenstein, 1985). Due to gaps and alignment problems, 1325 nucleotides were used in the calculation of identities between 16S rRNA sequences.

RESULTS

Phenotypic data

Strains CIP 105469^T (type strain of *P. gessardii*) and CIP 105470^T (type strain of *P. migulae*) are motile by means of single polar flagella (Fig. 1). The phenotypic characteristics of mineral-water strains studied in this work have been published previously (Verhille *et al.*, 1997).

Fig. 1. Electron micrographs of cells of *P. gessardii* CIP 105469^T (a) and *P. migulae* CIP 105470^T (b) showing the single polar flagella. Bars, 1 μm.

Table 2. DNA–DNA hybridization values between *P. migulae* CIP 105470^T, *P. gessardii* CIP 105469^T and phenotypically related species

Phenotypic clusters are as given by Verhille *et al.* (1997). Strains CFML 95-326 and CFML 95-323 do not belong to *P. migulae*. CFML, Collection de la Faculté de Médecine, Lille, France; CIP, Collection de l'Institut Pasteur, Paris, France. RBR, Relative binding ratio; –, not determined.

Strain	Phenotypic subcluster	Labelled DNA				G + C content (mol %)
		CIP 105470 ^T		CIP 105469 ^T		
		RBR (%)	ΔT_m (°C)	RBR (%)	ΔT_m (°C)	
<i>P. migulae</i>						
CIP 105470 ^T (= CFML 95-321 ^T)	XIIIb	100	0	31	—	58
CFML 95-341	XIIIb	99	—	26	—	—
CFML 95-320	XIIIb	98	—	25	—	—
CFML 95-318	XIIIb	97	0	26	—	—
CFML 95-316	XIIIb	88	—	27	—	59
CFML 95-333	XIIIb	88	—	27	—	—
CFML 95-327	XIIIb	87	—	23	—	—
CFML 95-317	XIIIb	87	1	28	—	—
CFML 95-324	XIIIb	83	2	25	—	58
CFML 95-263	XIIIb	74	2	21	—	—
CFML 95-326	XIIIb	61	13	56	10	—
CFML 95-323	XIIIb	56	9	26	—	—
<i>P. gessardii</i>						
CIP 105469 ^T (= CFML 95-251 ^T)	XVa	28	—	100	0	58
CFML 95-244	XVa	—	—	94	—	—
CFML 95-332	XVc	32	—	90	—	—
CFML 95-258	XVa	—	—	88	—	—
CFML 95-338	XVc	33	—	82	1	—
CFML 95-250	XVa	—	—	81	—	—
CFML 95-256	XVa	—	—	78	—	—
CFML 95-264	XVa	16	—	77	—	—
CFML 95-254	XVa	—	—	76	—	58
CFML 95-342	XVc	—	—	75	—	58
CFML 95-245	XVa	23	—	75	1	—
CFML 95-331	XVc	—	—	73	1	—
CFML 95-255	XVa	—	—	71	2	—

DNA–DNA hybridization

The DNA-binding ratios between the type strains of *P. gessardii* CIP 105469^T (= CFML 95-251^T, phenotypic subcluster XVa), *P. migulae* CIP 105470^T (= CFML 95-321^T, phenotypic subcluster XIIIb) and 65 isolates from mineral waters are given in Tables 2 and 3.

When the DNA of *P. gessardii* CIP 105469^T was used as the labelled probe, levels of reassociation with DNA from 13 strains from phenotypic subclusters XVa and XVc ranged from 71 to 100%. The ΔT_m values measured within this genomic group were 2 °C or less (Table 2). DNA–DNA similarity levels between strain CIP 105469^T and other strains from mineral waters included in the previously described clusters or subclusters (IX, X, XI, XIIe, XIIIa, XIIIb, XIIIc and

XIIId) ranged from 20 to 59% (Tables 2 and 3). The ΔT_m values obtained for strains from mineral waters that gave DNA similarity values of 50–69% to strain CIP 105469^T were 6 °C or more. Finally, DNA–DNA hybridizations between strain CIP 105469^T and other previously described species of the genus *Pseudomonas* yielded hybridization values below 49% (Table 4).

The levels of DNA–DNA hybridization between *P. migulae* CIP 105470^T and the other 11 isolates belonging to the same subcluster, XIIIb, ranged from 56 to 100%. Ten strains of this subcluster were 74–100% related to strain CIP 105470^T, with ΔT_m values of 2 °C or less (Table 2). The two remaining strains of subcluster XIIIb, CFML 95-326 and CFML 95-323, gave only low DNA similarity levels (61 and 56%,

Table 3. DNA–DNA hybridization values between *P. gessardii* CIP 105469^T, *P. migulae* CIP 105470^T and other strains isolated from mineral waters

Phenotypic clusters and subclusters are as given by Verhille *et al.* (1997). RBR, Relative binding ratio; –, not determined.

Strain	Labelled DNA			
	CIP 105469 ^T (= CFML 95-251 ^T)		CIP 105470 ^T (= CFML 95-321 ^T)	
	RBR (%)	ΔT_m (°C)	RBR (%)	ΔT_m (°C)
Phenotypic cluster IX				
CFML 95-307	23	–	39	–
CFML 95-311	36	–	19	–
CFML 95-312	39	–	38	–
CFML 95-306	35	–	29	–
CFML 95-308	33	–	17	–
CFML 95-303	26	–	50	9
CFML 95-309	43	–	19	–
CFML 95-305	36	–	15	–
CFML 95-304	20	–	11	–
CFML 95-322	25	–	30	–
Phenotypic cluster X				
CFML 95-285	33	–	46	–
CFML 95-280	22	–	51	9
CFML 95-281	25	–	54	10
Phenotypic cluster XI				
CFML 95-325	59	8	35	–
CFML 95-319	52	9	29	–
CFML 95-340	38	–	34	–
CFML 95-270	36	–	49	–
CFML 95-329	35	–	41	–
Phenotypic subcluster XIIe				
CFML 95-335	57	8	31	–
CFML 95-334	40	–	30	–
CFML 95-330	37	–	31	–
CFML 95-339	36	–	44	–
CFML 95-275	32	–	41	–
CFML 95-336	27	–	27	–
CFML 95-337	24	–	42	–
Phenotypic subcluster XIIIa				
CFML 95-313	33	–	26	–
CFML 95-302	36	–	16	–
CFML 95-314	28	–	28	–
CFML 95-310	35	–	20	–
Phenotypic subcluster XIIIc				
CFML 95-260	31	–	48	–
CFML 95-259	41	–	16	–
CFML 95-246	40	–	26	–
CFML 95-243	37	–	24	–
CFML 95-261	38	–	18	–
CFML 95-315	38	–	16	–
CFML 95-249	31	–	43	–
CFML 95-262	40	–	22	–
CFML 95-257	43	–	49	–
Phenotypic subcluster XIId				
CFML 95-282	58	6	37	–
CFML 95-271	23	–	46	–

respectively) with strain CIP 105470^T, which were confirmed by ΔT_m values of 13 and 9 °C. When hybridized with strain CIP 105470^T, the relative binding ratios with strains from mineral waters belonging to other phenotypic groups (IX, X, XI, XIIe, XIIIa, XIIIc and XIId) were less than 54 %, with a ΔT_m range of 9–10 °C (Tables 2 and 3). Finally, DNA–DNA similarity levels between strain CIP 105470^T and fluorescent or non-fluorescent species belonging or related to the genus *Pseudomonas* varied from 9 to 55 % (Table 4).

Hybridizations were also performed between the two new species, represented by strains CIP 105469^T and CIP 105470^T. Results are listed in Table 2 and varied from 16 to 33 %.

DNA G + C content

The G + C contents of DNA from strains *P. gessardii* CIP 105469^T, CFML 95-324, *P. migulae* CIP 105470^T, CFML 95-254 and CFML 95-342 were 58 mol %. The value calculated for strain CFML 95-316 was 59 mol % (Table 2).

Analysis of sequence data and phylogenetic analysis

16S rDNA sequences of 1516 nucleotides were determined for strains *P. gessardii* CIP 105469^T and *P. migulae* CIP 105470^T by sequencing PCR-amplified DNAs. Percentage identities between strains CIP 105470^T and CIP 105469^T and type strains of the *Pseudomonas* species listed by Kersters *et al.* (1996) were in the range 94.4–99.6 %. The closest neighbours of strain CIP 105469^T according to 16S rDNA sequences were *P. rhodesiae* CIP 104664^T and *P. veronii* CIP 104663^T (99.0 % for both) and also *Pseudomonas mucidolens* IAM 12406^T and *Pseudomonas synxantha* IAM 12356^T (99.6 % for both). As for strain CIP 105470^T, the highest values were obtained with *Pseudomonas corrugata* ATCC 29736^T (99.2 %) and *P. veronii* CIP 104663^T (99.3 %). The phylogenetic tree is displayed in Fig. 2.

DISCUSSION

A total of 25 strains isolated from natural mineral waters and identified phenotypically as fluorescent pseudomonads were previously clustered into the three phenotypic subclusters XIIIb, XVa and XVc (Verhille *et al.*, 1997). Additional genomic studies were necessary to unravel the taxonomic position of these subclusters within the genus *Pseudomonas sensu stricto* (Palleroni, 1984). According to Wayne *et al.* (1987), the phylogenetic definition of a species generally includes strains with 'approximately 70 % or greater DNA–DNA relatedness and with 5 °C or less ΔT_m '. Therefore, DNA–DNA hybridization experiments performed in this work with strains CFML 95-251^T

Table 4. Levels of DNA relatedness between *P. gessardii* CIP 105469^T, *P. migulae* CIP 105479^T and other strains of the genus *Pseudomonas*

Relative binding at 60 °C is shown. Culture collections not given in Table 1 are abbreviated as follows: CCEB, Culture Collection of Entomogenous Bacteria, Institute of Entomology, Czechoslovak Academy of Sciences, Prague, Czech Republic; CCM, Czechoslovak Collection of Microorganisms, University of Brno, Czech Republic; CFBP, Collection Française de Bactéries Phytopathogènes, INRA, Angers, France; CFML, Collection de la Faculté de Médecine de Lille, Lille, France.

Source of unlabelled DNA	Source of labelled DNA	
	CIP 105469 ^T	CIP 105470 ^T
<i>P. aeruginosa</i> ATCC 10145 ^T	9	30
<i>P. aeruginosa</i> ATCC 27853	27	24
<i>P. aeruginosa</i> ATCC 15692	20	15
<i>P. fluorescens</i> biovar I ATCC 13525 ^T	31	40
<i>P. fluorescens</i> biovar I ATCC 17397	25	20
<i>P. fluorescens</i> biovar I ATCC 17563	39	41
<i>P. fluorescens</i> biovar II ATCC 17482	45	31
<i>P. fluorescens</i> biovar II ATCC 17815	40	40
<i>P. fluorescens</i> biovar II ATCC 17816	49	29
<i>P. fluorescens</i> biovar II DSMZ 50106	33	40
<i>P. fluorescens</i> biovar III ATCC 17559	46	29
<i>P. fluorescens</i> biovar III ATCC 17571	36	41
<i>P. fluorescens</i> biovar III ATCC 17400	23	25
<i>P. fluorescens</i> biovar IV DSMZ 50415	40	44
<i>P. fluorescens</i> biovar IV ATCC 12983	25	45
<i>P. fluorescens</i> biovar V DSMZ 50148	28	55*
<i>P. fluorescens</i> biovar V ATCC 17386	21	35
<i>P. fluorescens</i> biovar V ATCC 17573	34	40
<i>P. fluorescens</i> biovar V ATCC 14150	37	35
<i>P. fluorescens</i> biovar V ATCC 15916	43	33
<i>P. fluorescens</i> biovar V ATCC 17518	40	35
<i>P. putida</i> biovar A ATCC 12633 ^T	14	45
<i>P. putida</i> biovar A DSMZ 50208	15	24
<i>P. putida</i> biovar B ATCC 17430	28	45
<i>P. putida</i> biovar B CCUG 1317	20	19
<i>P. putida</i> biovar B ATCC 17484	35	54*
<i>P. monteilii</i> CIP 104883 ^T	10	15
<i>P. rhodesiae</i> CIP 104664 ^T	36	22
<i>P. veronii</i> CIP 104663 ^T	33	25
<i>P. flavescentis</i> CIP 104204 ^T	38	11
<i>P. flavescentis</i> CIP 104205	35	11
<i>P. marginalis</i> ATCC 10844 ^T	29	30
<i>P. marginalis</i> DSMZ 50275	43	31
<i>P. marginalis</i> DSMZ 50276	44	34
<i>P. chlororaphis</i> DSMZ 50083 ^T	29	30
<i>P. chlororaphis</i> ATCC 9447	39	42
<i>P. chlororaphis</i> ATCC 17414	32	46
<i>P. aureofaciens</i> CCEB 518 ^T	24	34
<i>P. aureofaciens</i> ATCC 17415	45	33
<i>P. fuscovaginae</i> NCPPB 3085 ^T	20	27
<i>P. lundensis</i> CCM 573 ^T	14	42
<i>P. lundensis</i> CCUG 18758	16	24
<i>P. syringae</i> ATCC 19310 ^T	10	18
<i>P. savastanoi</i> CFBP 1670 ^T	8	46
<i>P. savastanoi</i> CFBP 2088	21	30
<i>P. savastanoi</i> CFBP 1838	16	20
<i>P. viridiflava</i> ATCC 13223 ^T	24	20

Table 4 (cont.)

Source of unlabelled DNA	Source of labelled DNA	
	CIP 105469 ^T	CIP 105470 ^T
<i>P. cichorii</i> DSMZ 50259 ^T	20	28
<i>P. agarici</i> ATCC 25941 ^T	18	48
<i>P. asplenii</i> ATCC 23835 ^T	23	23
<i>P. caricapapayae</i> NCPPB 1873 ^T	16	37
<i>P. tolaasii</i> NCPPB 2192 ^T	40	33
<i>P. tolaasii</i> NCPPB 1616	44	41
<i>P. stutzeri</i> ATCC 17588 ^T	26	12
<i>P. stutzeri</i> ATCC 17587	14	12
<i>P. stutzeri</i> ATCC 17591	14	12
<i>P. stutzeri</i> ATCC 17686	34	14
<i>P. mendocina</i> ATCC 25411 ^T	19	13
<i>P. mendocina</i> ATCC 25412	32	46
<i>P. alcaligenes</i> ATCC 14909 ^T	38	19
<i>P. pseudoalcaligenes</i> ATCC 17440 ^T	28	9
<i>P. pseudoalcaligenes</i> ATCC 12815	15	17
<i>P. fragi</i> ATCC 4973 ^T	12	23
<i>P. fragi</i> ATCC 27362	18	14
<i>P. mucidolens</i> CIP 103298 ^T	32	33
<i>P. synxantha</i> CIP 5922 ^T	26	23
<i>P. corrugata</i> ATCC 29736 ^T	15	14

* The unrelatedness of *P. fluorescens* biovar IV strain DSMZ 50148, *P. putida* biovar B strain ATCC 17484 and CIP 105470^T was confirmed by ΔT_m values of 13 °C.

and CFML 95-321^T and members of phenotypic groups XIIIb, XVa and XVc delineated two homogeneous groups.

When the DNA of strain CFML 95-251^T was used as the labelled probe, 13 strains, including members of both subclusters XVa and XVc, formed a genotypically homogeneous group, showing DNA similarity values of 71–100 %. Even if these strains did not belong to the same phenotypic subcluster, subclusters XVa and XVc are closely related since they were included in the same phenotypic cluster, XV (Verhille *et al.*, 1997). The ΔT_m values calculated for hybrids were 2 °C and less, supporting a high level of relatedness between these strains.

Ten of 12 strains of phenotypic subcluster XIIIb shared a high level of DNA relatedness (74–100 %) with DNA of strain CFML 95-321^T, used as the labelled probe. The ΔT_m values within this genomic group were 2 °C or less, indicating that these organisms were closely related. Only two strains from this phenotypic subcluster, CFML 95-326 and CFML 95-323, showed low DNA similarities with CFML 95-321^T. They were consequently removed from the genomic group. These two strains were phenotypically very similar to members of subcluster XIIIb, although two phenotypic characteristics were found to differentiate these strains from the others. Neither of these strains was able to utilize *N*-acetyl D-glucosamine or tri-carballylate as carbon sources. Furthermore, the

inability to assimilate 2-keto-D-gluconate differentiated strain CFML 95-323 from all other members of phenotypic subcluster XIIIb. Reciprocal DNA hybridization values obtained between the two above groups ranged from 16 to 33 %, indicating that they formed two separate genomic groups.

In conclusion, levels of hybridization between strains CFML 95-321^T and CFML 95-251^T and other members of the genus *Pseudomonas* were low. The highest values obtained were between *Pseudomonas fluorescens* biovar V strain DSMZ 50148 and *Pseudomonas putida* biovar B strain ATCC 1784 and CFML 95-321^T (55 and 34 %, respectively). Their unrelatedness was confirmed by a ΔT_m value of 13 °C.

According to the above results, the strains that formed these two genomic groups were sufficiently closely related to warrant recognition as members of two separate species, for which we propose the names *P. gessardii* sp. nov. (type strain CIP 105469^T = CFML 95-251^T) and *P. migulae* sp. nov. (type strain CIP 105470^T = CFML 95-321^T).

Ideally, the species should be phenotypically distinct, so that species definition can be performed easily. Fourteen phenotypic features were found to differentiate the two new species from each other. *P. gessardii* strains assimilated L-arabitol, xylitol, myo-inositol, adonitol and meso-erythritol. On the other hand, *P. migulae* strains utilized the following com-

Fig. 2. Phylogenetic position of *P. gessardii* and *P. migulae* within the genus *Pseudomonas*, derived from 16S rRNA sequences. Numbers at branch-points represent percentage frequencies at which given branches appeared in 1000 bootstrap replications. Evolutionary distance is represented only in the horizontal direction. Strains indicated by * and † belong to phenotypic cluster IX of Verhille *et al.* (1997).

pounds as carbon sources: L-arabinose, D-xylose, D-saccharate, *meso*-tartrate, tricarballoylate, D-glucuronate, D-galacturonate, phenylacetate and histamine. Variable characteristics among strains of *P. gessardii* and *P. migulae* are given in Tables 5 and 6, respectively. The two new species could also be differentiated from *P. veronii* (Elomari *et al.*, 1997), *P. rhodesiae* (Coroler *et al.*, 1996) and *P. libanensis* (Dabboussi *et al.*, 1999) and from other *Pseudomonas* species (Palleroni, 1984) by various phenotypic characteristics listed in Table 7.

The highest 16S rDNA identities (99% and more) measured between *P. gessardii* CIP 105469^T, *P. migulae* CIP 105470^T and *Pseudomonas* type strains listed by Kersters *et al.* (1996) were not supported by high DNA–DNA hybridization levels (less than 36%, see Table 4). In fact, DNA–DNA hybridization remains the optimal method for measuring the degree of relatedness between closely related organisms (Fox *et al.*, 1992; Stackebrandt & Goebel, 1994). However, the phylogenetic tree displayed in Fig. 2 clearly demonstrates that *P. gessardii* CIP 105469^T and *P. migulae* CIP 105470^T belong to the '*Pseudomonas fluorescens*

intragenetic cluster', as it has been described by Moore *et al.* (1996). On the basis of the above physiological, biochemical and genotypic characteristics, we describe the following two new species.

Description of *Pseudomonas gessardii* sp. nov.

Pseudomonas gessardii (ges.sar'di.i. M.L. gen. masc. n. *gessardii* after C. Gessard, who isolated '*Bacterium aeruginosum*' for the first time and studied its pigment).

Cells are asporogeneous, rod-shaped and stain Gram-negative. Cells are motile by means of one polar flagellum. Pyoverdine is produced on King B medium and on S1 medium (Gould *et al.*, 1985). No phenazine pigments are observed on King A medium. Cells are oxidase- and catalase-positive. Cetrinide (*N*-acetyl-*N,N,N*-trimethylammonium) is assimilated. Significant growth is observed only at low salt concentrations (up to 0.8%) and not at 5 or 7%. Strains grow at 4 °C and not at 41 °C. Levan is produced from sucrose. Starch and *o*-nitrophenyl β-D-galactopyranoside cannot be assimilated. Does not form indole from tryptophan. Does not decarboxylate lysine or ornithine.

Table 5. Variable characteristics among 13 strains of *P. gessardii*

Characteristic	Reaction of majority of strains*	Reaction of CIP 105469 ^T
Conventional tests		
Nitrate reduction, tetrathionate reductase	+(1)	+
Growth on 3% NaCl	+(1)	—
Growth on 2,3,5-triphenyltetrazolium chloride	—(1)	—
Malonate assimilation, denitrification, Tween esterase	+(2)	+
Gelatin liquefaction, urease, haemolysin	—(2)	—
Lecithinase production	+(3)	+
Tributyryl esterase	+(5)	—
Carbon sources		
Tryptamine, D-mannose	+(1)	+
DL- α -Amino- <i>n</i> -valerate	+(2)	—
D-Malate	—(2)	—
Gentisate, mucate	+(3)	+
<i>m</i> -Hydroxybenzoate, benzoate	+(4)	+
<i>p</i> -Hydroxybenzoate	+(4)	—
D-Galactose	+(5)	+
Ethanolamine	+(6)	—
Itaconate	+(2)	+
<i>trans</i> -Aconitate	—(1)	—
Enzymic tests		
<i>N</i> -Acetyl- β -D-glucosaminidase, <i>N</i> -carboxybenzoxy-arginine-4-methoxy-arylamidase	—(5)	—
Esterase-C4, esterase-C12, esterase-C14, α -L-glutamate arylamidase, L-ornithine arylamidase, L-serine arylamidase, L-lysyl-L-alanine arylamidase, L-alanyl-L-phenylalanyl-L-proline arylamidase, L-alanyl-L-phenylalanyl-L-prolyl-L-alanine arylamidase, L-seryl-L-methionine arylamidase, L-phenylalanyl-L-arginine arylamidase, L-phenylalanyl-L-prolyl-L-alanine arylamidase	+(1)	+
S-Benzyl-cysteine arylamidase, L-histidyl-L-leucyl-L-histidine arylamidase	—(1)	—
Esterase-C16, L-prolyl-L-arginine arylamidase, L-lysyl-L-serine-4-methoxy arylamidase	—(2)	—
L-Threonine arylamidase, L-histidyl-L-serine arylamidase, L-leucyl-L-alanine arylamidase, L-lysyl-L-lysine arylamidase, L-histidyl-L-phenylalanine arylamidase, L-valyl-L-tyrosyl-L-serine arylamidase	+(2)	+
L-Arginyl-L-arginine arylamidase	+(3)	+
L-Tyrosine arylamidase	—(4)	—
Leucyl-glycine arylamidase, L-isoleucine arylamidase	+(4)	+
L-Leucyl-L-leucyl-L-valyl-L-tyrosyl-L-serine arylamidase	—(4)	+
L-Histidine arylamidase, L-tryptophan arylamidase	+(5)	+
L-Aspartate arylamidase	+(5)	—
L-Hydroxyproline arylamidase	—(6)	+
Glycyl-proline arylamidase	+(6)	+
α -L-Glutamyl- α -L-glutamic arylamidase, L-phenylalanyl-L-proline arylamidase	—(6)	—
α -L-Glutamyl-L-histidine arylamidase	+(6)	+
Glycyl-L-tryptophan arylamidase	—(6)	+

* Numbers in parentheses are the numbers of strains differing from the more common result.

Table 6. Variable characteristics among 10 strains of *P. migulae*

Characteristic	Reaction of majority of strains*	Reaction of CIP 105470 ^T
Conventional tests		
Denitrification	+(1)	+
Urease, haemolysin	-(1)	-
Nitrate reduction, growth on 3% NaCl, malonate assimilation	c (5)	+
Tributylin esterase	+(2)	+
Carbon sources		
D-Tartrate, 3-phenylpropionate	-(1)	-
D-Malate, DL- α -amino- <i>n</i> -valerate	+(1)	+
α -Ketoglutarate	+(2)	+
Enzymic tests		
<i>N</i> -Acetyl- β -D-glucosaminidase, γ -glutamyltransferase, tyrosyl-L-serine arylamidase, L-phenylalanyl-L-arginine arylamidase, L-phenylalanyl-L-prolyl-L-alanine arylamidase	+(1)	+
Esterase-C5, esterase-C9, esterase-C10	+(1)	-
Esterase-C12, glycyl-proline arylamidase, α -L-glutamate arylamidase, α -L-glutamyl-L-histidine arylamidase	-(1)	-
L-Pyrrolidone arylamidase	-(2)	-
L-Alanyl-L-phenylalanyl-L-proline arylamidase	+(2)	+
L-Alanyl-L-phenylalanyl-L-prolyl-L-alanine arylamidase	+(2)	-
α -L-Aspartyl-L-arginine arylamidase	-(2)	+
L-Tyrosine arylamidase, L-alanyl-L-arginine arylamidase	+(3)	+
<i>S</i> -Benzyl-cysteine arylamidase	-(3)	+
<i>N</i> -Carboxybenzoxy-L-arginine-4-methoxy arylamidase	+(3)	-
Esterase-C4	+(4)	-
L-Aspartate arylamidase	-(4)	+
L-Isoleucine arylamidase	+(4)	+
Glycyl-L-arginine arylamidase, L-phenyl-L-proline arylamidase	-(4)	-
L-Hydroxyproline arylamidase, L-threonine arylamidase, L-lysyl-L-lysine arylamidase	c (5)	+

* The numbers in parentheses are the numbers of strains differing from the more common result; c, 50% of strains are positive.

No elastase, coagulase, β -xylosidase (*p*-nitrophenyl β -D-xylopyranoside test) or phenylalanine deaminase activities are reported. No activity against DNA is noticed. Strains exhibit no acetyl-methyl-carbinol (acetoin) activity. Fibrinolysin test is negative.

All strains utilize the following substrates as carbon and energy sources: D-glucose, D-fructose, D-trehalose, D-ribose, D-arabitol, L-arabitol, xylitol, glycerol, *myo*-inositol, D-mannitol, adonitol, *meso*-erythritol, L-malate, *cis*-aconitate, citrate, 2-keto-D-gluconate, *N*-acetyl D-glucosamine, D-gluconate, protocatechuate, quinate, betaine, putrescine, DL- α -amino-*n*-butyrate, DL-lactate, caprate, caprylate, succinate, fumarate, glutarate, DL-glycerate, D-glucosamine, itaconate, DL- β -hydroxybutyrate, L-aspartate, L-glutamate, L-proline, D-alanine, L-alanine, L-serine, malonate, propionate, L-tyrosine and 2-oxoglutarate. The following

compounds are not utilized: L-sorbose, D-melibiose, sucrose, D-raffinose, maltotriose, maltose, α -lactose, lactulose, 1-*O*-methyl β -galactopyranoside, 1-*O*-methyl α -galactopyranoside, D-cellobiose, β -gentiobiose, 1-*O*-methyl β -D-glucopyranoside, aesculin, L-arabinose, D-xylose, palatinose, α -L-rhamnose, α -L-fucose, D-melezitose, dulcitol, D-tagatose, maltitol, D-turanose, D-sorbitol, hydroxyquinoline β -glucuronide, D-lyxose, 1-*O*-methyl α -D-glucopyranoside, 3-*O*-methyl D-glucopyranose, D-saccharate, mucate, L-tartrate, D-tartrate, *meso*-tartrate, tricarballylate, D-glucuronate, D-galacturonate, 5-keto-D-gluconate, L-tryptophan, phenylacetate, 3-phenylpropionate, *m*-coumarate, trigonelline, histamine and L-histidine.

Strains have the following enzyme activities: esterases C5, C6, C8, C9 and C10, L-pyrrolidone arylamidase, L-phenylalanine arylamidase, L-lysine arylamidase, gly-

Table 7. Characteristics that differentiate *P. gessardii* and *P. migulae* from other *Pseudomonas* strains

—, At least 90 % of strains negative; d, 11–89 % of strains positive; +, at least 90 % strains positive; ND, not determined.

Characteristic	<i>P. gessardii</i>	<i>P. migulae</i>	<i>P. aeruginosa</i>	<i>P. fluorescens</i> biovars					<i>P. chlororaphis</i>	<i>P. putida</i> biovars		<i>P. veronii</i>	<i>P. rhodesiae</i>	<i>P. libanensis</i>
				I	II	III	IV	V		A	B			
Pyocyanin production	—	—	+	—	—	—	—	—	—	—	—	—	—	—
Denitrification	d	+	+	—	+	+	+	—	+	—	—	+	—	—
Growth at 4 °C	+	+	—	+	+	+	+	d	+	d	+	+	+	+
Growth at 41 °C	—	—	+	—	—	—	—	—	—	—	—	—	—	—
Production of:														
Lecithinase	d	+	—	+	d	+	+	d	+	—	—	—	+	+
Levan	+	+	—	+	+	—	+	—	+	—	—	+	+	+
Gelatin liquefaction	—	—	+	+	+	+	+	+	+	—	—	d	—	ND
Tween esterase	d	—	+	d	—	d	d	d	+	d	d	—	—	—
Assimilation of:														
myo-Inositol	+	—	—	d	+	d	+	d	+	—	—	+	+	+
Mucate	d	+	—	+	+	d	+	+	+	d	+	d	+	+
Phenylacetate	—	—	—	—	—	d	—	d	d	d	+	—	—	—
L-Arabinose	—	+	—	+	+	d	+	d	—	d	+	+	+	+
D-Trehalose	+	+	—	+	+	d	+	d	+	—	—	d	+	d
D-Xylose	—	+	—	+	d	d	d	d	—	d	d	+	+	+
D-Saccharate	—	+	—	+	+	d	+	d	+	+	+	ND	ND	+
meso-Tartrate	—	+	—	—	—	d	—	d	—	d	—	—	—	d
meso-Erythritol	+	—	—	d	d	+	—	d	—	—	—	+	—	+
Adonitol	+	—	—	+	—	d	—	d	—	—	—	—	—	+
D-Glucuronate	—	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+
Tryptamine	+	—	—	—	d	d	—	—	—	d	+	—	—	—
Itaconate	d	—	+	+	d	d	—	d	+	d	—	ND	ND	+
α-Aminobutyrate	+	+	—	—	—	—	—	—	—	—	d	+	+	+
L-Histidine	—	—	+	+	d	+	+	d	+	+	+	d	+	—
Xylitol	+	—	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+
L-Tryptophan	—	—	d	+	d	d	—	d	+	—	+	+	—	—
Histamine	—	+	+	d	—	d	—	d	d	d	+	—	—	—
Trigonelline	—	—	—	d	d	d	—	d	—	d	+	+	—	—
Tricarballoylate	—	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	—
Benzoate	d	+	+	d	d	d	+	d	+	d	+	—	—	—
D-Mannose	+	+	—	+	+	+	+	d	+	d	d	+	+	+
D-Alanine	+	+	+	+	+	+	+	—	+	+	+	+	+	+
Ethanolamine	d	+	d	+	d	d	+	d	d	d	d	+	+	+
D-Galacturonate	—	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+
D-Sorbitol	—	—	—	+	+	d	+	d	—	—	d	+	+	+
D-Galactose	d	+	—	+	+	d	+	d	d	—	d	+	+	+
Sucrose	—	—	—	+	+	—	+	d	+	—	d	+	—	—
D-Malate	d	+	d	—	d	d	+	d	d	d	d	—	—	ND
L-Tartrate	—	—	—	—	—	—	+	—	d	d	d	—	—	ND
L-Arabitol	+	—	ND	ND	ND	ND	ND	ND	ND	ND	ND	—	—	+

cine arylamidase, L-arginine arylamidase, L-alanine arylamidase, γ -glutamyltransferase, methionine arylamidase, glycyl-glycine arylamidase, glycyl-phenylalanine arylamidase, L-seryl-tyrosine arylamidase, L-glutamine arylamidase, L-proline arylamidase, β -alanine arylamidase, L-alanyl-L-arginine arylamidase, α -L-aspartyl-L-alanine arylamidase, α -L-aspartyl-L-arginine arylamidase, glycyl-L-alanine arylamidase, glycyl-L-arginine arylamidase and *N*-carboxybenzoxy-glycyl-glycyl-L-arginine arylamidase. None of the strains possess the following enzyme activities: α -D-galactosidase, β -D-galactosidase, phospho- β -D-galactosidase, α -L-arabinosidase, α -D-glucosidase, β -D-glucosidase, β -D-galacturonohydrolase, β -D-glucuronidase, α -maltosidase, β -maltosidase, *N*-acetyl- α -D-glucosaminidase, α -L-fucosidase, β -D-fucosidase, β -L-fucosidase, β -D-lactosidase, α -D-mannosidase, β -D-mannosidase, α -D-xylosidase, β -D-xylosidase, esterase-C18, *N*-benzoyl-leucine arylamidase, *N*-benzoyl-L-alanine-4-

methoxy arylamidase, *N*-carboxybenzoxy-arginyl-4-methoxy arylamidase, *N*-acetyl-glycyl-L-lysine arylamidase.

Temperatures for growth range from 4 to 35 °C with an optimum at 30 °C. Strains were isolated from natural mineral waters. No clinical significance is known.

Pseudomonas gessardii sp. nov. as described includes 13 strains: the type strain, CIP 105469^T (= CFML 95-251^T), and the following other strains: CFML 95-244, CFML 95-332, CFML 95-258, CFML 95-338, CFML 95-250, CFML 95-256, CFML 95-264, CFML 95-254, CFML 95-245, CFML 95-342, CFML 95-331 and CFML 95-255. The above strains have been deposited in the Collection de la Faculté de Médecine, Lille, France (CFML) and the Collection de l'Institut Pasteur, Paris, France (CIP).

Description of *Pseudomonas migulae* sp. nov.

Pseudomonas migulae (mi'gu.lae. M.L. gen. n. *migulae* after W. Migula, who defined the morphological properties of the genus *Pseudomonas*).

Colonies on nutrient agar are smooth and circular. Cells are Gram-negative and possess a single polar flagellum. Cells produce the characteristic fluorescent pigment on King B medium and on S1 medium (Gould *et al.*, 1985) and possess catalase and oxidase activities. No phenazine pigments are produced on King A medium. Strains tolerate low salt concentrations (up to 0.8%) but higher concentrations (5 and 7%) inhibit growth. The 2,3,5-triphenyltetrazolium chloride, fibrinolysin and haemolysin tests are negative. No tetrathionate reductase or Tween esterase activities are reported. The Voges-Proskauer reaction (acetoin) is negative. Strains do not liquefy gelatin. *o*-Nitrophenyl β -D-galactopyranoside is not assimilated. Starch is not hydrolysed. Levan is produced from sucrose and strains possess an arginine decarboxylase activity. Cetrimide (*N*-acetyl *N,N,N*-trimethylammonium) and L-tyrosine can be used for growth. Lysine and ornithine catabolism do not occur. Cells have no elastase, DNase, coagulase, phenylalanine deaminase, lecithinase or β -xylosidase (*p*-nitrophenyl β -D-galactopyranoside) activities. No indole is formed from tryptophan. Growth occurs at 4 °C but is invariably negative at 41 °C.

All strains utilize the following substrates as carbon and energy sources: D-glucose, D-fructose, D-galactose, D-trehalose, D-mannose, D-ribose, L-arabinose, D-xylose, D-arabitol, glycerol, D-mannitol, D-saccharate, mucate, *meso*-tartrate, L-malate, *cis*-aconitate, *trans*-aconitate, tricarballoylate, citrate, D-glucuronate, D-galacturonate, *N*-acetyl D-glucosamine, 2-keto-D-gluconate, D-gluconate, phenylacetate, protocatechuate, *p*-hydroxybenzoate, quinate, betaine, benzoate, putrescine, DL- α -amino-*n*-butyrate, histamine, DL-lactate, caprate, caprylate, succinate, fumarate, glutarate, DL-glycerate, ethanolamine, D-glucosamine, DL- β -hydroxybutyrate, L-aspartate, L-glutamate, L-proline, D-alanine, L-alanine, L-serine, malonate, propionate and L-tyrosine. The following compounds are not utilized: L-sorbose, D-melibiose, sucrose, D-raffinose, maltotriose, maltose, α -lactose, lactulose, 1-*O*-methyl β -galactopyranoside, 1-*O*-methyl α -galactopyranoside, D-cellobiose, β -gentiobiose, 1-*O*-methyl β -D-glucopyranoside, aesculin, palatinose, α -L-rhamnose, α -L-fucose, D-melezitose, L-arabitol, xylitol, dulcitol, D-tagatose, *myo*-inositol, maltitol, D-turanose, D-sorbitol, adonitol, hydroxyquinoline β -glucuronide, D-lyxose, *meso*-erythritol, 1-*O*-methyl α -D-glucopyranoside, 3-*O*-methyl D-glucopyranose, L-tartrate, 5-keto-D-gluconate, L-tryptophan, gentisate, *m*-hydroxybenzoate, *m*-coumarate, trigonelline, L-histidine, tryptamine and itaconate.

All strains have the following enzyme activities: esterase-C6, esterase-C8, L-phenylalanine arylamidase, L-lysine arylamidase, L-histidine aryl-

amidase, glycine arylamidase, L-arginine arylamidase, L-alanine arylamidase, methionine arylamidase, glycyl-glycine arylamidase, glycyl-phenylalanine arylamidase, leucyl-glycine arylamidase, L-seryl-L-tyrosine arylamidase, L-glutamine arylamidase, L-ornithine arylamidase, L-proline arylamidase, L-serine arylamidase, L-tryptophan arylamidase, β -alanine arylamidase, α -L-aspartyl-L-alanine arylamidase, glycyl-L-alanine arylamidase, glycyl-L-tryptophan arylamidase, L-leucyl-L-alanine arylamidase, L-seryl-L-methionine arylamidase, *N*-carboxybenzoxy-glycyl-glycyl-L-arginine arylamidase and L-histidyl-L-phenylalanine arylamidase. None of the strains possess the following enzymic activities: α -D-galactosidase, β -D-galactosidase, phospho- β -D-galactosidase, α -L-arabinosidase, α -D-glucosidase, β -D-glucosidase, β -D-galacturonohydrolase, β -D-glucuronidase, α -maltosidase, β -maltosidase, *N*-acetyl- α -D-glucosaminidase, α -L-fucosidase, β -D-fucosidase, β -L-fucosidase, β -D-lactosidase, α -D-mannosidase, β -D-mannosidase, α -D-xylosidase, β -D-xylosidase, esterase-C14, esterase-C16, esterase-C18, *N*-benzoyl-leucine arylamidase, L-arginyl-L-arginine arylamidase, α -L-glutamyl- α -L-glutamyl arylamidase, L-histidyl-L-leucyl-L-histidine arylamidase, L-histidyl-L-serine arylamidase, L-leucyl-L-leucyl-L-valyl-L-tyrosyl-L-serine arylamidase, L-prolyl-L-arginine arylamidase, L-valyl-L-tyrosyl-L-serine arylamidase, *N*-benzoyl-L-alanine-4-methoxy arylamidase, *N*-acetyl-glycyl-L-lysine arylamidase and L-lysyl-L-serine-4-methoxy arylamidase.

Temperatures for growth range from 4 to 35 °C, with an optimum at 30 °C. Strains examined in this study were isolated exclusively from natural mineral waters. No clinical significance is known.

The following 10 strains are included in *Pseudomonas migulae* sp. nov.: the type strain, CIP 105470^T (= CFML 95-321^T), CFML 95-341, CFML 95-320, CFML 95-318, CFML 95-327, CFML 95-316, CFML 95-333, CFML 95-324, CFML 95-317 and CFML 95-263. The above strains have been deposited in the Collection de la Faculté de Médecine, Lille, France (CFML) and the Collection de l'Institut Pasteur, Paris, France (CIP).

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