Pseudomonas grimontii sp. nov.

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The vernacular name 'fluorescent *Pseudomonas* group 97-514' was coined for a group of 43 strains isolated from two French natural mineral waters. All these strains were Gram-negative, rod-shaped and motile by means of a single polar flagellum. They produced fluorescent pigment (pyoverdine) on King B medium, catalase and cytochrome oxidase. They were capable of respiratory but not fermentative metabolism. They were not able to accumulate poly- β -hydroxybutyrate and possessed an arginine dihydrolase system. DNA–DNA relatedness studies (S1 nuclease method) showed that the 43 strains of 'fluorescent *Pseudomonas* group 97-514' formed a genetically homogeneous group (DNA–DNA relatedness ranged from 70 to 100%). A total of 76 strains representing well-known or partially characterized species of the genus *Pseudomonas sensu stricto* had 7–56% DNA hybridization with strain CFML 97-514^T. The highest DNA binding values were found with *Pseudomonas veronii* CIP 104663^T (52%), *Pseudomonas rhodesiae* CIP 104664^T (56%), *Pseudomonas marginalis* ATCC 10844^T (56%), *Pseudomonas gessardii* CIP 105469^T (53%) and *Pseudomonas cedrella* CIP 105541^T (52%). Their unrelatedness was confirmed by ΔT_m values greater than 7 °C. On the basis of the results of phenotypic and DNA–DNA hybridization studies, a novel *Pseudomonas* species, *Pseudomonas grimontii* sp. nov., is proposed for the 43 strains of 'fluorescent *Pseudomonas* group 97-514'. The type strain is strain CFML 97-514^T (= CIP 106645^T = ATCC BAA-140^T). The G+C content of the DNA of the type strain was 58 mol%. A comparison of the complete 16S rRNA gene sequence of the type strain CFML 97-514^T and the sequence of other strains of the genus *Pseudomonas* revealed that the novel species fell within the '*Pseudomonas fluorescens* intrageneric cluster'. Members of *P. grimontii* grew at 4 °C but not at 41 °C. They were able to use D-xylose, α -L-rhamnose, α -aminobutyrate, meso-erythritol and itaconate as sole sources of carbon and energy and formed levan from sucrose. Strains do not possess lecithinase or Tween esterase activities. The clinical significance of *P. grimontii* is unknown.

Keywords: *Pseudomonas grimontii* sp. nov., polyphasic taxonomy, mineral waters

INTRODUCTION

In recent years, *Pseudomonas* strains have been studied with increasing interest because of their importance in medical, food and environmental microbiology and phytopathology. The purpose of our research group is the taxonomic study of 'fluorescent' *Pseudomonas* strains isolated from natural mineral waters. In recent years, we have described seven novel species isolated

from natural mineral waters: *Pseudomonas rhodesiae* (Coroler *et al.*, 1996), *Pseudomonas veronii* (Elomari *et al.*, 1996), *Pseudomonas jessenii* and *Pseudomonas mandelii* (Verhille *et al.*, 1999a), *Pseudomonas gessardii* and *Pseudomonas migulae* (Verhille *et al.*, 1999b) and *Pseudomonas brenneri* (Baïda *et al.*, 2001).

The vernacular name 'fluorescent *Pseudomonas* group 97-514' was coined for 43 strains isolated from two natural mineral waters and not identified as any of our previously described species. All strains were able to hydrolyse starch, which is an unusual property among fluorescent *Pseudomonas* (Palleroni, 1984), except

The EMBL accession number for the 16S rRNA gene sequence of *P. grimontii* CFML 97-514^T is AF268029.

Fig. 1. Electron micrograph of a cell of *P. grimontii* sp. nov. CFML 97-514^T showing the single polar flagellum. Bar, 0.5 µm.

the recently described species *P. brenneri* (Baïda *et al.*, 2001). The outcome of this polyphasic taxonomic study is the description of a novel species, *Pseudomonas grimontii* sp. nov.

METHODS

Bacterial strains. The 119 bacterial strains used in this study (Tables 2 and 3) included 43 wild strains isolated from two French natural mineral waters after bottling and identified as 'fluorescent *Pseudomonas*' and 76 type or collection strains from official collections, which were used for control purposes; they were chosen as representatives of authentic *Pseudomonas* species. All bacteria were cultured on Mueller–Hinton medium at 30 °C.

Flagellation study. Negative staining was done with fixed cells of the type strain of *P. grimontii* sp. nov., CFML 97-514^T, according to the method described by Hoeniger (1965).

Table 1. Characteristics that differentiate *P. grimontii* sp. nov. from various members of *Pseudomonas sensu stricto*

Taxa: 1, *P. grimontii* sp. nov.; 2, *Pseudomonas aeruginosa*; 3, *P. fluorescens* biovar I; 4, *P. fluorescens* biovar II; 5, *P. fluorescens* biovar III; 6, *P. fluorescens* biovar IV; 7, *P. fluorescens* biovar V; 8, *Pseudomonas chlororaphis*; 9, *Pseudomonas putida* biovar A; 10, *P. putida* biovar B; 11, *P. veronii*; 12, *P. rhodesiae*; 13, *P. gessardii*; 14, *P. migulae*; 15, *P. mandelii*; 16, *P. jessenii*; 17, *P. brenneri*; 18, *Pseudomonas libanensis*; 19, *P. cedrella*; 20, *P. orientalis*. Characteristics are scored as: –, ≥ 90% of strains negative; +, ≥ 90% of strains positive; d, 11–89% of strains positive; ND, not determined. Data were taken from Palleroni (1984), Elomari *et al.* (1996), Coroler *et al.* (1996), Verhille *et al.* (1999a, b), Dabboussi *et al.* (1999a, b) and Baïda *et al.* (2001).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Pyocyanin production	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Denitrification	+	+	–	+	+	+	–	+	–	–	+	–	d	+	+	–	+	–	d	–
Growth at 4 °C	+	–	+	+	+	+	d	+	d	+	+	+	+	+	+	+	+	+	+	+
Growth at 41 °C	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lecithinase	–	–	+	d	+	+	d	+	–	–	–	+	d	+	–	–	+	+	–	d
Starch hydrolysis	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Levan	+	–	+	+	–	+	–	+	–	–	+	+	+	+	+	+	+	+	+	+
Assimilation of:																				
D-Ribose	+	+	+	+	d	+	d	+	d	d	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	–	+	d	d	d	d	–	d	d	+	+	–	+	+	d	–	+	+	+
α-L-Rhamnose	+	–	–	d	d	–	d	–	–	–	–	–	–	–	d	d	+	d	d	d
D-Mannose	+	–	+	+	+	+	d	+	d	d	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	d	+	d	+	d	d	+	+	+	+	+	+	+	+	+	+
D-Trehalose	+	–	+	+	d	+	d	+	–	–	d	+	+	+	+	d	+	d	+	+
2-Keto-D-gluconate	+	+	+	+	+	+	+	+	d	+	+	+	+	+	d	+	+	+	+	+
Mucate	+	–	+	+	d	+	+	+	d	+	d	+	d	+	+	+	+	+	+	+
Malonate	+	+	+	+	d	+	d	+	d	+	+	+	+	+	+	+	+	+	+	+
D-Tartrate	d	–	–	d	–	–	d	–	d	d	–	–	–	–	–	–	–	–	d	–
meso-Tartrate	–	–	–	–	d	–	d	–	d	–	–	–	–	+	+	+	–	d	–	–
m-Hydroxybenzoate	d	–	–	–	–	–	–	d	d	d	–	–	d	–	d	d	–	–	–	–
α-Aminobutyrate	+	–	–	–	–	–	–	–	–	d	+	+	+	+	+	+	+	+	+	+
meso-Erythritol	+	–	d	d	+	–	d	–	–	–	+	–	+	–	–	–	+	+	+	–
D-Sorbitol	+	–	+	+	d	+	d	–	–	d	+	+	–	–	+	d	–	+	+	+
myo-Inositol	+	–	d	+	d	+	d	+	–	–	+	+	+	–	+	d	+	+	+	+
Adonitol	–	–	+	–	d	–	d	–	–	–	–	–	+	–	d	d	+	+	+	+
Benzoate	d	+	d	d	d	+	d	+	d	+	+	–	d	+	+	+	+	–	–	–
Itaconate	+	+	+	d	d	–	d	+	d	–	ND	ND	d	–	d	d	+	+	–	d
L-Histidine	–	+	+	d	+	+	d	+	+	+	d	+	–	–	–	–	–	–	–	–
L-Tryptophan	–	d	+	d	d	–	d	+	–	+	+	–	–	–	–	–	–	–	–	–
Histamine	–	+	d	–	d	–	d	d	d	+	–	–	–	+	+	+	–	–	–	–
Tryptamine	–	–	–	d	d	–	–	–	d	+	–	–	+	–	–	–	d	–	–	–
Trigonelline	+	–	d	d	d	–	d	–	d	+	+	–	+	–	–	+	–	–	+	+

The stained bacteria were examined with a JEOL type 100 CX transmission electron microscope.

Phenotypic characterization. Forty-two conventional tests were performed as utilized by Gavini *et al.* (1989). Carbon source utilization tests were studied using the Biotype-100 system (bioMérieux) as described by Grimont *et al.* (1996). Enzymic tests were performed on API ZYM strips (bioMérieux). The enzymic activities tested included four esterase, seven peptidase and eight oxidase activities. These enzymic activities were studied at 30 °C for 4 h. The tests were scored according to the manufacturer's recommendations.

DNA extraction. Strains were grown on Mueller–Hinton medium plates. Chromosomal DNA was extracted as described previously (Marmur, 1961; Beji *et al.*, 1987). 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 & Doty (1962).

DNA–DNA hybridization. Native DNA was labelled *in vitro* by nick translation with tritium-labelled nucleotides (Grimont *et al.*, 1980). The S1 nuclease/trichloroacetic acid method for hybridization has been described previously (Crosa *et al.*, 1973; Grimont *et al.*, 1980). Each hybridization experiment was at least done twice.

Thermal stability of duplexes. The thermal stability of duplexes was determined by using the method of Crosa *et al.* (1973). The divergence between DNAs was estimated by determining the ΔT_m value.

DNA base composition. G + C content was calculated from the thermal denaturation curve by using the equation of De Ley (1970).

16S rDNA sequence determination. The almost complete 16S rRNA gene sequence was determined for strain CFML 97-514^T by direct PCR sequencing. DNA was amplified by using two 16S rRNA universal primers, pA and pH, described previously (Edwards *et al.*, 1989). PCR amplification were performed as described by Bařda *et al.* (2001).

Phylogenetic analysis. Using the program CLUSTAL X, which is a major up-date of the multiple alignment program CLUSTAL W (Thompson *et al.*, 1994), the primary structure was aligned and compared with the primary structures of reference strains of the genus *Pseudomonas sensu stricto* (Kerstens *et al.*, 1996) obtained from the EMBL sequence database. Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura, 1980) and a phylogenetic tree was constructed by the neighbour-joining method of Saitou & Nei (1987). The tree topology was tested by a bootstrap analysis of 1000 resamplings (Felsenstein, 1985).

Nucleotide sequence accession numbers. The strain designations and accession numbers of 16S rRNA gene sequences of other species that were compared with the CFML 97-514^T sequence in this study are displayed in Fig. 2.

RESULTS AND DISCUSSION

Single polar flagella (Fig. 1) were observed by scanning electron microscopy on cells of *Pseudomonas grimontii* CFML 97-514^T. A summary of phenotypic properties is shown in Table 1 and in the description of the novel species.

Hybridization experiments performed with the labelled DNA of *P. grimontii* CFML 97-514^T and the 42

Table 2. DNA–DNA hybridization values between *P. grimontii* CFML 97-514^T and other mineral water isolates

Labelled DNA from strain CFML 97-514^T was used. All strains were isolated from two French natural mineral waters (A and B) after bottling. CFML, Collection de la Faculté de Médecine, Lille, France; RBR, relative binding ratio.

Strain	Origin of mineral water	RBR (%) [ΔT_m (°C)]
CFML 97-514 ^T	B	100 [0]
CFML 97-492	B	99
CFML 97-498	B	99
CFML 97-505	B	97
CFML 97-528	B	96
CFML 97-521	B	96
CFML 97-520	B	96
CFML 97-525	B	96
CFML 97-493	B	96
CFML 97-509	B	96
CFML 97-485	B	95
CFML 97-501	B	95
CFML 97-508	B	95
CFML 97-510	B	95
CFML 97-523	B	95
CFML 97-490	B	94
CFML 97-483	B	94
CFML 97-530	B	94
CFML 97-484	B	94
CFML 97-491	B	93
CFML 97-486	B	92
CFML 97-515	B	91
CFML 97-517	B	91
CFML 97-489	B	88
CFML 97-507	B	87
CFML 97-512	B	87
CFML 97-519	B	87
CFML 97-467	A	86
CFML 96-331	A	85
CFML 97-506	B	85
CFML 97-504	B	84
CFML 97-494	B	84
CFML 97-503	B	83
CFML 97-592	B	82
CFML 97-495	B	81
CFML 97-502	B	79
CFML 97-481	B	78
CFML 97-524	B	78
CFML 96-323	A	75 [0]
CFML 96-347	A	75 [0]
CFML 96-336	A	75 [0]
CFML 97-500	B	73 [0]
CFML 97-516	B	70 [1]

other isolates of 'fluorescent *Pseudomonas* group 97-514' showed that strains were 70–100% related to CFML 97-514^T (Table 2). DNA–DNA hybridization values between strain CFML 97-514^T and other previously described species of the genus *Pseudomonas*

Table 3. Levels of DNA relatedness of *P. grimontii* CFML 97-514^T to different type and collection strains of the genus *Pseudomonas*

Labelled DNA from strain CFML 97-514^T was used. ATCC, American Type Culture Collection, Manassas, VA, USA; 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; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; CIP, Collection de l'Institut Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; NCPPB, National Collection of Plant-pathogenic bacteria, Harpenden, UK; CFBP, Collection Française des Bactéries Phytopathogènes, Angers, France.

Source of unlabelled DNA	RBR (%) [Δ <i>T</i> _m (°C)]	Source of unlabelled DNA	RBR (%) [Δ <i>T</i> _m (°C)]
<i>P. aeruginosa</i>		<i>P. savastanoi</i> (cont.)	
ATCC 10145 ^T	20	CFBP 1838	10
ATCC 27853	11	<i>Pseudomonas viridiflava</i> ATCC 13223 ^T	10
ATCC 15692	38	<i>Pseudomonas syringae</i> ATCC 19310 ^T	15
<i>P. fluorescens</i> biovar I		<i>Pseudomonas cichorii</i> DSM 50259 ^T	17
ATCC 13525 ^T	49	<i>Pseudomonas agarici</i> ATCC 25941 ^T	20
ATCC 17563	40	<i>Pseudomonas asplenii</i> ATCC 23835 ^T	23
<i>P. fluorescens</i> biovar II		<i>Pseudomonas caricapapayae</i> NCPPB 1873 ^T	19
CFBP 12301	16	<i>Pseudomonas tolaasii</i>	
ATCC 17482	33	NCPPB 2192 ^T	46
CFBP 11346	33	NCPPB 1616	49
CFBP 12296	40	<i>Pseudomonas stutzeri</i>	
CFBP 11759	15	ATCC 17588 ^T	10
CFBP 11776	16	ATCC 17587	11
<i>P. fluorescens</i> biovar III		ATCC 17591	28
ATCC 17559	19	ATCC 17686	39
ATCC 17571	36	<i>Pseudomonas mendocina</i>	
ATCC 17400	36	ATCC 25411 ^T	13
<i>P. fluorescens</i> biovar IV		ATCC 25412	10
DSM 50415	35	<i>Pseudomonas alcaligenes</i> ATCC 14909 ^T	10
ATCC 12983	31	<i>Pseudomonas pseudoalcaligenes</i>	
<i>P. fluorescens</i> biovar V		ATCC 17440 ^T	16
ATCC 15916	46	ATCC 12815	18
ATCC 17518	48	<i>Pseudomonas fragi</i>	
ATCC 17573	40	ATCC 4973 ^T	39
DSM 50148	27	ATCC 27362	16
ATCC 17386	25	<i>Pseudomonas mucidolens</i> CIP 103298 ^T	38
<i>P. putida</i> biovar A		<i>Pseudomonas ficuserectae</i> CFBP 3224 ^T	7
ATCC 12633 ^T	38	<i>Pseudomonas synxantha</i> CIP 5922 ^T	36
DSM 50208	21	' <i>Pseudomonas coronafaciens</i> ' CFBP 2216	10
<i>P. putida</i> biovar B		<i>Pseudomonas corrugata</i> CIP 105514 ^T	27
ATCC 17484	25	<i>Pseudomonas cannabina</i> CFBP 2341 ^T	18
ATCC 17430	24	<i>P. chlororaphis</i>	
CCUG 1317	20	DSM 50083 ^T	39
<i>P. veronii</i> CIP 104663 ^T	52 [10]	ATCC 9447	45
<i>P. rhodesiae</i> CIP 104664 ^T	56 [10]	ATCC 17414	30
<i>Pseudomonas monteilii</i> CIP 104883 ^T	35	<i>Pseudomonas aureofaciens</i>	
<i>P. jessenii</i> CIP 105274 ^T	18	CCEB 518 ^T	26
<i>P. mandelii</i> CIP 105273 ^T	29	ATCC 17415	31
<i>P. gessardii</i> CIP 105469 ^T	53 [11]	<i>Pseudomonas lundensis</i>	
<i>P. migulae</i> CIP 105470 ^T	32	CCM 573 ^T	19
<i>P. libanensis</i> CIP 105460 ^T	37	CCUG 18758	14
<i>P. orientalis</i> CIP 105540 ^T	36	<i>Pseudomonas flavescens</i>	
<i>P. cedrella</i> CIP 105541 ^T	52 [9]	CIP 104204 ^T	38
<i>Pseudomonas fuscovaginae</i> NCPPB 3085 ^T	16	CIP 104205	13
<i>Pseudomonas savastanoi</i>		<i>P. marginalis</i> ATCC 10844 ^T	56 [7]
CFBP 1670 ^T	15	<i>P. brenneri</i> CIP 106646 ^T	33
CFBP 2088	20

Fig. 2. Unrooted tree, constructed by the neighbour-joining method, showing the phylogenetic relationships of *P. grimontii* sp. nov. CFML 97-514^T and other species of the genus *Pseudomonas sensu stricto*. Numbers at nodes show the level of bootstrap support based on data for 1000 replications. Bar, 0.01 substitutions per nucleotide position.

varied from 7 to 56 %, and their unrelatedness was confirmed by ΔT_m values greater than 7 °C (Table 3). The DNA G+C content of *Pseudomonas grimontii* CFML 97-514^T was 58 mol %.

The phylogenetic tree based on 16S rDNA sequencing is displayed in Fig. 2. Percentage identities between strain CFML 97-514^T and other *Pseudomonas* species ranged from 93 to 99 %. The highest similarity (99 %) was found with the five strains *P. rhodesiae* CIP

104664^T, *P. veronii* CIP 104663^T, *Pseudomonas orientalis* CIP 105540^T, *Pseudomonas marginalis* LMG 2210^T and *P. brenneri* CIP 106646^T, but they were clearly different in DNA–DNA hybridization (Table 3) and phenotypic characteristics (Table 1).

According to the our results, we conclude that members of ‘fluorescent *Pseudomonas* group 97-514’ belong to a novel species, for which we propose the name *P. grimontii* sp. nov. The novel species can be differ-

entiated from other 'fluorescent' *Pseudomonas* species by several phenotypic features (Table 1).

Description of *Pseudomonas grimontii* sp. nov.

Pseudomonas grimontii (gri.mon'ti.i. N.L. gen. n. *grimontii* of Grimont, in honour of the French bacteriologist P. A. D. Grimont, for all his work in the field of bacterial taxonomy).

Cells are Gram-negative, asporogenous, rod-shaped and motile by means of a single polar flagellum (Fig. 1). Colonies are smooth on nutrient agar, circular, non-pigmented and haemolytic (98 % of strains) when grown on blood agar. All strains produce a fluorescent pigment on both King B medium and S1 medium (Gould *et al.*, 1985). Arginine dihydrolase, tributyrin esterase (93 % of strains), catalase and oxidase are also produced. Lysine and ornithine are not decarboxylated. Cells can reduce nitrate to nitrite and can grow at low salt concentrations (0.8 %) but not at high salt concentrations (5 or 7 %). Strains do not possess any DNase, coagulase, tetrathionate reductase (95 % of strains), elastase or Tween esterase activities. Fibrinolysis and acetyl-methylcarbinol (acetoin) tests are negative. Acid is formed from starch. Poly- β -hydroxybutyrate is not accumulated.

At least 90 % of strains utilize the following substrates as carbon and energy sources: α -D-glucose, β -D-fructose, D-galactose, L-arabinose, sucrose, D-arabitol, glycerol, D-glucosamine, D-saccharate, L-malate, *cis*-aconitate, citrate, D-glucuronate, D-galacturonate, 5-keto-D-glucuronate, *N*-acetyl D-glucosamine, D-glucuronate, protocatechuate, *p*-hydroxybenzoate, quinate, betaine, putrescine, DL-lactate, caprate, caprylate, succinate, fumarate, glutarate, DL-glycerate, 3-hydroxybutyrate, L-aspartate, L-glutamate, L-proline, D-alanine, L-alanine, L-serine, propionate, L-tyrosine and 2-oxoglutarate. The following compounds are not utilized by 90 % or more of the strains: L-sorbose, α -D-melibiose, maltotriose, D-raffinose, maltose, α -lactose, lactulose, 1-*O*-methyl β -galactopyranoside, 1-*O*-methyl α -galactopyranoside, D-cellobiose, β -gentiobiose, 1-*O*-methyl β -D-glucopyranoside, palatinose, α -L-fucose, D-melezitose, L-arabitol, xylitol, dulcitol, D-tagatose, maltitol, D-turanose, hydroxyquinoline β -glucuronide, 1-*O*-methyl α -D-glucopyranoside, 3-*O*-methyl D-glucopyranose, L-tartrate, *trans*-aconitate, tricarallylate, phenylacetate, gentisate, 3-phenylpropionate and *m*-coumarate. The other characteristics are displayed in Table 1.

At least 90 % of strains possess the following enzyme activities: esterase C₄, alkaline phosphatase, esterase lipase C₈ and leucine arylamidase. At least 90 % of strains do not possess the following enzyme activities: trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, β -glucuronidase, α -glucosidase, α -mannosidase and α -fucosidase.

All strains have been isolated from natural mineral water. No clinical significance is known. The type

strain is CFML 97-514^T (= CIP 106645^T = ATCC BAA-140^T).

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