NOTE

***Pseudomonas lini* sp. nov., a novel species from bulk and rhizospheric soils**

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The taxonomic position of eight fluorescent *Pseudomonas* strains isolated from bulk and rhizospheric soils, and from water was examined. These eight strains clustered in one phenon together with *Pseudomonas mandelii* (CFBP 4844^T), but could still be differentiated from this type strain by four phenotypic features. The eight stains exhibited internal DNA–DNA hybridization values ranging from 60 to 100%, with ΔT_m below 5 °C (3.9 and 4.3 °C) for the lowest values (60 and 66%). The percentages of hybridization with type or reference strains of other *Pseudomonas* species tested ranged from 12 to 60% (ΔT_m = 5.5 °C), indicating that the eight isolates studied constituted a discrete DNA homology group. Comparison of the 16S rDNA sequence of the strain representing this group (CFBP 5737^T) with the sequences of other strains belonging to the genus *Pseudomonas* revealed that strain CFBP 5737^T was a member of this genus and that these bacteria did not cluster with any previously described species of the genus *Pseudomonas*. The eight isolates belonged to two siderovars different from those described so far. On the basis of the results of phenotypic, DNA–DNA and phylogenetic analyses, and of siderotyping, a new species, *Pseudomonas lini* sp. nov. (type strain CFBP 5737^T) is proposed.

Keywords: *Pseudomonas lini* sp. nov., soil, rhizosphere, phenotypic, genotypic analysis

The genus *Pseudomonas sensu stricto* belongs to the γ -subclass of the *Proteobacteria* (Woese, 1987) and contains mostly fluorescent *Pseudomonas* spp. as well as a few non-fluorescent species. The recent changes in the classification of pseudomonads are given by Kersters *et al.* (1996) and Anzai *et al.* (2000). Fluorescent *Pseudomonas* strains constitute the most important group among authentic pseudomonads and can be distinguished from other pseudomonads by their ability to produce water-soluble yellow-green pigments, pyoverdines (PVDs), which act as siderophores for these bacteria (Meyer *et al.*, 1987). This group of fluorescent pseudomonads includes mainly saprophytic and phytopathogenic species and a few human- or animal-pathogenic species. The great diversity within saprophytic species has been illustrated by extensive studies (Laguerre *et al.*, 1994; Latour *et*

al., 1996; Lemanceau *et al.*, 1995; Palleroni *et al.*, 1973), resulting in the recent description of many new species (Achouak *et al.*, 2000; Andersen *et al.*, 2000; Behrendt *et al.*, 1999; Bennasar *et al.*, 1996; Dabboussi *et al.*, 1999a, b; Elomari *et al.*, 1996, 1997; Gardan *et al.*, 1999; Hildebrand *et al.*, 1994; Nishimori *et al.*, 2000; Verhille *et al.*, 1999a, b).

Saprophytic fluorescent pseudomonads are ubiquitous. They are abundant in various environments, such as water and soil environments. The ecological significance of these bacteria has been shown many times (Holloway, 1992; Lemanceau, 1992; Weller, 1988). Because of their importance, numerous ecological studies have already been conducted on these bacteria (Frey *et al.*, 1997; Johnsen & Nielsen, 1999; Latour *et al.*, 1999; Lemanceau *et al.*, 1995; Raaijmakers & Weller, 1998; Rainey *et al.*, 1994). During such studies, investigators are often faced with difficulties in identifying bacteria isolated from natural environments (Bossis *et al.*, 2000).

When characterizing the phenotypic diversity of pop-

Abbreviation: PVD; pyoverdine.

The GenBank accession number for the partial 16S rDNA sequence of *Pseudomonas lini* CFBP 5737^T is AY035996.

Table 1. Strains used in this study

Abbreviation: ATCC, American Type Culture Collection, Manassas, VA, USA; CFBP, Collection Française de Bactéries Phytopathogènes, UMR de Pathologie Végétale, INRA-INH/Université, 49071 Beaucouzé cedex, France; CIP, Collection de l'Institut Pasteur, Paris, France; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; ICMP, International Collection of Microorganisms from Plants, Plant Disease Division, DSIR Mount Albert Centre, Auckland, New Zealand; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NCPPB, National Collection of Plant-pathogenic bacteria, Central Science Laboratory, Sand Hutton, York, UK.

Species	Strain	Other designation	Origin
<i>Pseudomonas aeruginosa</i>	CFBP 2466 ^T	ATCC 10145 ^T	Unknown
<i>Pseudomonas agarici</i>	CFBP 2063 ^T	ATCC 25941 ^T	<i>Agaricus bisporus</i>
<i>Pseudomonas asplenii</i>	CFBP 3279 ^T	ATCC 23835 ^T	<i>Asplenium nidus</i>
<i>Pseudomonas aureofaciens</i>	CFBP 2133 ^T	ATCC 13985 ^T	Clay in kerosene
<i>Pseudomonas brassicacearum</i>	CFBP 5593 ^T	DBK11 ^T	<i>Brassica napus</i>
' <i>Pseudomonas blatchfordae</i> '	CFBP 3280	NCPPB 3374	<i>Phaseolus vulgaris</i>
' <i>Pseudomonas cedrella</i> '	CFBP 4839	CIP 105541	Spring waters
<i>Pseudomonas chlororaphis</i>	CFBP 2132 ^T	ATCC 9446 ^T	Plate contaminant
<i>Pseudomonas chlororaphis</i>	CFBP 5760	DTR133	Rhizospheric soil of Dijon (<i>Lycopersicon esculentum</i>)
<i>Pseudomonas cichorii</i>	CFBP 2101 ^T	ATCC 10857 ^T	<i>Cichorium endivia</i>
<i>Pseudomonas corrugata</i>	CFBP 2431 ^T	ATCC 29736 ^T	<i>Lycopersicon esculentum</i>
<i>Pseudomonas flectens</i>	CFBP 3281 ^T	ATCC 12775 ^T	<i>Phaseolus vulgaris</i>
<i>Pseudomonas fluorescens</i> bv. I	CFBP 2102 ^T	ATCC 13525 ^T	Water reservoir
<i>Pseudomonas fluorescens</i> bv. I	CFBP 2123	ATCC 17397	Tap water
<i>Pseudomonas fluorescens</i> bv. II	CFBP 2125	ATCC 17482	Unknown
<i>Pseudomonas fluorescens</i> bv. II	CFBP 5757	CTR212	Rhizospheric soil of Châteaurenard (<i>Lycopersicon esculentum</i>)
<i>Pseudomonas fluorescens</i> bv. II	CFBP 5758	CTR1015	Rhizospheric soil of Châteaurenard (<i>Lycopersicon esculentum</i>)
<i>Pseudomonas fluorescens</i> bv. II	CFBP 5759	C7R12	<i>Linum usitatissimum</i>
<i>Pseudomonas fluorescens</i> bv. III	CFBP 2127	ATCC 17400	Chicken egg
<i>Pseudomonas fluorescens</i> bv. V	CFBP 2130	ATCC 17386	Water
<i>Pseudomonas fluorescens</i> bv. VI	CFBP 2392	A6	<i>Phaseolus vulgaris</i>
<i>Pseudomonas fluorescens</i> bv. VI	CFBP 5755	CLR711	Rhizospheric soil of Châteaurenard (<i>Linum usitatissimum</i>)
<i>Pseudomonas fluorescens</i> bv. VI	CFBP 5756	CTRp112	Rhizospheric soil of Châteaurenard (<i>Lycopersicon esculentum</i>)
<i>Pseudomonas fuscovaginae</i>	CFBP 2065 ^T	ICMP 5940 ^T	<i>Oryza sativa</i>
<i>Pseudomonas gessardii</i>	CFBP 4840 ^T	CIP 105469 ^T	Natural mineral waters
<i>Pseudomonas jessenii</i>	CFBP 4842 ^T	CIP 105274 ^T	Natural mineral waters
<i>Pseudomonas libanensis</i>	CFBP 4841 ^T	CIP 105460 ^T	Spring waters
<i>Pseudomonas lini</i>	CFBP 2129	ATCC 17513	Water
<i>Pseudomonas lini</i>	CFBP 5732	CS611	Bulk soil of Châteaurenard
<i>Pseudomonas lini</i>	CFBP 5733	CLRp812	Rhizospheric soil of Châteaurenard (<i>Linum usitatissimum</i>)
<i>Pseudomonas lini</i>	CFBP 5734	CLE513	Rhizospheric soil of Châteaurenard (<i>Linum usitatissimum</i>)
<i>Pseudomonas lini</i>	CFBP 5735	DLR426	Rhizospheric soil of Dijon (<i>Linum usitatissimum</i>)
<i>Pseudomonas lini</i>	CFBP 5736	DLRp214	Rhizospheric soil of Dijon (<i>Linum usitatissimum</i>)
<i>Pseudomonas lini</i>	CFBP 5737 ^T	DLE411J ^T	Rhizospheric soil of Dijon (<i>Linum usitatissimum</i>)
<i>Pseudomonas lini</i>	CFBP 5738	DTR335	Rhizospheric soil of Dijon (<i>Lycopersicon esculentum</i>)
<i>Pseudomonas mandelii</i>	CFBP 4844 ^T	CIP 105273 ^T	Natural mineral waters
<i>Pseudomonas marginalis</i> pv. <i>alfalfae</i>	CFBP 2039	NCPPB 2644	<i>Medicago sativa</i>
<i>Pseudomonas marginalis</i> pv. <i>marginalis</i>	CFBP 3300 ^T	ATCC 10844 ^T	<i>Cichorium intybus</i>
<i>Pseudomonas marginalis</i> pv. <i>pastinacea</i>	CFBP 2038	ATCC 13889	<i>Pastinaca sativa</i>
<i>Pseudomonas migulae</i>	CFBP 4843 ^T	CIP 105470 ^T	Natural mineral waters
<i>Pseudomonas monteilii</i>	CFBP 4845 ^T	CIP 104883 ^T	Clinical source

Table 1 (cont.)

Species	Strain	Other designation	Origin
' <i>Pseudomonas mosselii</i> '	CFBP 4846 ^T	CIP 105274 ^T	Mineral water
' <i>Pseudomonas orientalis</i> '	CFBP 4863	CIP 105540	Spring waters
' <i>Pseudomonas pavonacea</i> '	CFBP 5038	IAM 1155	Creamery waste
<i>Pseudomonas putida</i> bv. A	CFBP 2066 ^T	ATCC 12633 ^T	Soil
<i>Pseudomonas putida</i> bv. A	CFBP 5039	NCIMB 9816	Garden soil
<i>Pseudomonas putida</i> bv. A	CFBP 5739	CS111	Bulk soil of Châteaurenard
<i>Pseudomonas putida</i> bv. A	CFBP 5740	CS413	Bulk soil of Châteaurenard
<i>Pseudomonas putida</i> bv. A	CFBP 5741	DS131	Bulk soil of Dijon
<i>Pseudomonas putida</i> bv. A	CFBP 5742	DS1026	Bulk soil of Dijon
<i>Pseudomonas putida</i> bv. A	CFBP 5743	DLR223	Rhizospheric soil of Dijon (<i>Linum usitatissimum</i>)
<i>Pseudomonas putida</i> bv. A	CFBP 5744	DLR228	Rhizospheric soil of Dijon (<i>Linum usitatissimum</i>)
<i>Pseudomonas putida</i> bv. A	CFBP 5745	DLE3216	Rhizospheric soil of Dijon (<i>Linum usitatissimum</i>)
<i>Pseudomonas putida</i> bv. A	CFBP 5746	DTRp621	Rhizospheric soil of Dijon (<i>Lycopersicon esculentum</i>)
<i>Pseudomonas putida</i> bv. B	CFBP 3140	ATCC 17430	Unknown
<i>Pseudomonas putida</i> bv. B	CFBP 5030	ATCC 17484	Soil
<i>Pseudomonas rhodesiae</i>	CFBP 4305 ^T	CIP 104664 ^T	Mineral water
' <i>Pseudomonas salomonii</i> '	CFBP 2022 ^T		<i>Allium sativum</i>
<i>Pseudomonas</i> sp.	CFBP 5747	DS824	Bulk soil of Dijon
<i>Pseudomonas</i> sp.	CFBP 5748	101.3	<i>Lycopersicon esculentum</i>
<i>Pseudomonas</i> sp.	CFBP 5749	101.62	<i>Lycopersicon esculentum</i>
<i>Pseudomonas</i> sp.	CFBP 5750	TSNII	<i>Lycopersicon esculentum</i>
<i>Pseudomonas</i> sp.	CFBP 5751	PsB	Rhizospheric soil (pinyon-juniper)
<i>Pseudomonas</i> sp.	CFBP 5752	PsF	Rhizospheric soil (pinyon-juniper)
<i>Pseudomonas</i> sp.	CFBP 5753	PsK	Rhizospheric soil (pinyon-juniper)
<i>Pseudomonas thivervalensis</i>	CFBP 5754 ^T	SBK26 ^T	<i>Arabidopsis thaliana</i>
<i>Pseudomonas tolaasii</i>	CFBP 2068 ^T	ATCC 33618 ^T	<i>Agaricus bisporus</i>
<i>Pseudomonas veronii</i>	CFBP 4304 ^T	CIP 104663 ^T	Natural mineral waters

ulations of fluorescent pseudomonads in different soil environments (bulk and rhizospheric soils), Latour *et al.* (1996) identified 21 groups among the 340 isolates analysed. One strain of each group was subsequently characterized together with reference and type strains by a polyphasic taxonomic approach. Based on the data yielded, a new species, *Pseudomonas lini* sp. nov., is proposed for eight of the strains included in a phenotypic cluster. Seven of these strains are representative of 66 isolated from different soil environments and one was isolated from water.

Bacterial strains

A total of 68 strains of *Pseudomonas* spp. were used in this study (Table 1): (i) 21 strains were previously isolated from soils of Châteaurenard and Dijon (France) cultivated with flax (*Linum usitatissimum* L., cv. opaline) or tomato (*Lycopersicon esculentum* Mill., cv. H63-5) (rhizospheric soils), or uncultivated (bulk soils) (Latour *et al.*, 1996); and (ii) 19 reference strains plus 28 type strains specifically included for numerical taxonomy and for DNA–DNA hybridizations.

Numerical taxonomy

A total of 119 characteristics, based on the results of the 20 conventional biochemical tests as indicated by Sutra *et al.* (1997) and on the ability of the bacteria to

assimilate 99 carbon sources, obtained with Biotype 100 strips (bioMérieux), as recommended by the manufacturer, were included in the numerical taxonomy analysis. The matrix of similarities was calculated using the Jaccard coefficient (Sneath & Sokal, 1973). The similarity values were converted into distances (distance = 1 – % of similarity) and cluster analysis was performed using the unweighted pair group method with averages. At a given distance level and for the different phenons, the amount of information for each test was measured by calculating the coefficient of diagnostic ability to determine the discriminating biochemical characteristics (Descamps & Veron, 1981).

A dendrogram showing phenotypic distances between the 68 strains included in this study is presented in Fig. 1. At a distance level of 0.2048, 11 phenons and 8 isolated phenotypes were identified (Fig. 1). Strains clustered in phenons and isolated strains could be discriminated with at least two tests (Table 2). Phenon 2 included 9 strains: the type strain of *Pseudomonas mandelii* and eight strains of the novel species *Pseudomonas lini* isolated from soil environments and from water. Strains contained in phenon 2 could be differentiated from those of the most related phenon (phenon 1) by three characters (D-lyxose, trigonelline and D-xylose). Within phenon 2, *Pseudomonas mande-*

Fig. 1. Dendrogram of phenotypic distances for 68 strains.

lii CFBP 4844^T, although sharing many traits with *Pseudomonas lini*, could still be discriminated from this species by four tests (assimilation of histamine, sucrose, L-histidine and *meso*-tartrate) (Table 2).

Siderotyping

Culture conditions for PVD production, isoelectric pH (pI) determination of PVD isoforms through isoelec-

trofocusing (IEF) and PVD-mediated ⁵⁹Fe incorporation were performed as described by Munsch *et al.* (2000). Strains belonging to phenon 2 could be grouped into three siderovars according to IEF and uptake data (Table 3). The third siderovar only included *Pseudomonas mandelii* CFBP 4844^T and was clearly distinguishable from the two other siderovars grouping *Pseudomonas lini* strains.

Table 2. Phenotypic characteristics that differentiate phenons 1–11 and isolated strains

+, 90–100 % of the strains positive; –, 90–100 % of the strains negative; D, 11–89 % of the strains positive. The numbers in parentheses are the percentages of positive strains.

Characteristic	Phenon no.											Isolated strain*								
	1	2	2†	3	4	5	6	7	8	9	10	11	a	b	c	d	e	f	g	h
Assimilation of:‡																				
D-Sorbitol	+	+	+	+	+	—	D (33)	—	—	—	—	—	+	+	—	—	—	—	—	—
myo-Inositol	+	+	+	+	+	—	—	—	—	+	+	+	+	—	—	—	+	—	—	—
Histamine	—	—	+	D (50)	—	+	+	D (29)	+	+	—	+	—	—	+	+	—	—	—	—
D-Trehalose	+	+	+	+	+	—	—	D (86)	—	+	+	D (33)	+	+	—	—	—	+	—	—
D-Xylose	+	—	—	D (50)	D (86)	—	D (67)	D (71)	—	—	—	—	+	—	—	—	+	—	—	—
D-Galacturonate	+	D (89)	+	+	+	D (57)	D (67)	+	—	—	—	—	+	+	+	—	+	—	—	—
D-Glucuronate	+	D (63)	+	+	+	D (57)	D (67)	+	—	—	—	—	+	+	+	—	+	—	—	—
D-Lyxose	+	—	—	+	D (57)	—	—	D (14)	—	—	—	—	—	—	—	—	—	—	—	—
Sucrose	+	+	—	D (50)	D (57)	D (14)	—	D (43)	—	+	—	+	+	+	+	+	—	+	—	+
L-Arabinose	+	+	+	+	D (86)	+	+	+	D (50)	D (40)	—	—	+	+	—	—	+	+	—	—
Adonitol	—	—	—	—	+	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—
Trigonelline	+	—	—	D (50)	D (29)	D (71)	+	D (86)	D (50)	—	—	+	+	—	+	—	+	—	—	—
Phenylacetate	—	—	—	D (50)	D (14)	D (86)	+	D (14)	D (50)	D (80)	—	—	—	—	+	—	—	—	—	—
L-Tartrate	—	D (33)	—	—	—	D (86)	—	—	—	D (20)	—	+	—	—	+	—	+	—	—	—
L-Histidine	D (17)	+	—	—	D (14)	D (86)	—	D (43)	—	D (40)	D (50)	+	—	+	—	—	—	+	—	—
Propionate	+	D (78)	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	—	+	—
D-Glucosamine	+	D (75)	+	+	+	D (71)	+	+	+	+	+	D (33)	+	+	+	+	—	—	—	—
Gentisate	—	—	—	+	—	—	—	—	—	D (20)	D (50)	—	—	—	—	+	—	—	—	—
m-Hydroxybenzoate	—	—	—	+	—	D (14)	—	—	—	D (20)	D (50)	—	—	—	—	—	—	—	—	—
meso-Tartrate	D (15)	—	+	D (50)	D (86)	D (57)	D (33)	D (71)	+	D (20)	—	D (67)	—	—	+	—	+	—	—	—
Levan production	+	D (25)	—	—	D (29)	—	—	—	—	D (80)	—	—	+	—	—	—	—	—	—	+
Nitrate reduction	+	+	+	—	D (43)	D (43)	+	—	—	D (60)	+	—	—	+	+	+	—	+	—	—
Gelatin hydrolysis	D (75)	+	+	+	D (71)	—	—	D (86)	+	+	D (50)	—	+	—	—	+	—	+	—	+
Tween 80 hydrolysis	D (67)	D (75)	+	—	D (43)	D (86)	—	D (43)	+	+	+	+	+	—	—	+	—	+	—	—

* a, '*Pseudomonas orientalis*' CFBP 4863; b, *Pseudomonas fluorescens* bv. II CFBP 2125; c, *Pseudomonas putida* bv. A CFBP 2066^T; d, *Pseudomonas aeruginosa* CFBP 2466^T; e, *Pseudomonas cichorii* CFBP 2101^T; f, *Pseudomonas fluorescens* bv. II CFBP 5757; g, *Pseudomonas agarici* CFBP 2063^T; h, *Pseudomonas flectens* CFBP 3281^T.

† *Pseudomonas mandelii* CFBP 4844^T.

‡ Biotype 100 substrates.

Table 3. Siderotyping of the strains belonging to phenon 2

Species	Strain	PVD isoform pl values	Iron incorporation as mediated by:		
			PVD (5737 ^T) or PVD (2129)	PVD (5732)	PVD (4844 ^T)
<i>Pseudomonas lini</i>	CFBP 2129	7.55, 5.30, 5.20	+	—	—
<i>Pseudomonas lini</i>	CFBP 5737 ^T	7.55, 5.30, 5.20	+	—	—
<i>Pseudomonas lini</i>	CFBP 5732	5.25, 4.45	—	+	—
<i>Pseudomonas lini</i>	CFBP 5733	5.25, 4.45	—	+	—
<i>Pseudomonas lini</i>	CFBP 5734	5.25, 4.45	—	+	—
<i>Pseudomonas lini</i>	CFBP 5735	5.25, 4.45	—	+	—
<i>Pseudomonas lini</i>	CFBP 5736	5.25, 4.45	—	+	—
<i>Pseudomonas lini</i>	CFBP 5738	5.25, 4.45	—	+	—
<i>Pseudomonas mandelii</i>	CFBP 4844 ^T	9.2, 7.7	—	—	+

Sequencing of 16S rDNA and phylogenetic analysis

The 16S rRNA gene of strain CFBP 5737^T was amplified by PCR using the primer pair fD1 and rD1 derived from conserved regions of 16S rDNA genes and described by Weisburg *et al.* (1991). PCR amplification was performed in a total volume of 100 µl by mixing 5 µl bacterial cell suspension in a PCR mixture containing each of the four dNTPs at a concentration of 10 µM each, primers fD1 and rD1 at a concentration of 0.2 µM each, and 4 U *Taq* DNA polymerase. Amplifications were carried out in a thermal cycler using the following programme: initial denaturation for 3 min at 95 °C, 40 cycles of denaturation (1 min at 94 °C), annealing (1 min at 55 °C) and extension (2 min at 72 °C), and final extension (3 min at 72 °C). The 16S rRNA gene sequence of CFBP 5737^T was determined directly from PCR products in both the forward and reverse directions ('Gene-Walking') (Genome Express). The 16S rDNA sequence of strain CFBP 5737^T was aligned manually by reference to a database of about 20000 previously aligned bacterial 16S rDNA sequences.

Three phylogenetic methods were used to assess the phylogenetic position of the 16S rDNA sequence of strain CFBP 5737^T: the bioNJ algorithm (Gascuel, 1997), maximum-likelihood and maximum-parsimony (PHYLIP, Phylogeny Inference Package, version 3.573c, distributed by J. Felsenstein, Department of Genetics, University of Washington, Seattle, WA, USA). The robustness of each topology was checked using the neighbour-joining method and 500 bootstrap replications (Kimura two-parameter correction). Trees were drawn using the NJPLOT program (Perrière & Gouy, 1996). All analyses showed that strain CFBP 5737^T always clustered within the γ group, more precisely within the clade of the true *Pseudomonas* (data not shown). To obtain a precise phylogenetic topology, more detailed analyses, comprising sequences for every type strain of the *Pseudomonas* genus for which a 16S rDNA sequence was available, were conducted (Fig. 2). Domains used for these analyses were regions of the 16S rDNA sequences available for the 64 sequences studied (positions 86–1313 of CFBP 5737^T). The

phylogenetic tree shows that strain CFBP 5737^T falls within the radiation of the genus *Pseudomonas*. The basis of modern bacterial taxonomy relies mostly on a cladistic model: bacterial species grouped within a single genus or a single species descend from a common ancestor from whom no other species that is not within that cluster also descends (a clade). Although gene trees cannot always be equated to species trees (Slowinski & Page, 1999), gene trees derived from 16S rDNA sequences have so far been viewed as a good indicator of species trees and used accordingly in bacterial systematics. An important rule for analysing gene trees is to estimate not only the robustness of a topology, but also to ascertain whether the tree obtained is the 'true' tree. Since the consistency of any given method is only valid within limits (Felsenstein, 1988; Huelsenbeck & Hillis, 1993), a given method may produce a robust tree (high bootstrap numbers) that is not the true tree. One way to circumvent this problem is to compare different methods that rely on different models (Kim, 1993). It is unlikely that different methods, if they all produce a wrong tree, will produce the same tree. So, if all methods indicate the same topology, there is a good chance that the true tree has been obtained. By contrast when different trees are obtained, the true tree cannot be inferred from the data. The 16S rDNA sequence of strain CFBP 5737^T showed variable positions in the different dendrograms, and as this sequence never clustered with that of any known species, strain CFBP 5737^T could not be attributed to any recognized species.

The 16S rDNA sequence of CFBP 5737^T (accession no. AY035996) shows 98.9% similarity with that of *Pseudomonas mandelii* CFBP 4844^T (accession no. AF058286).

DNA–DNA hybridization

Extraction and purification of the DNAs were performed using methods described by Brenner *et al.* (1982). Native DNAs were labelled *in vitro* by random priming with tritium-labelled nucleotides (Megaprime DNA-labelling system; Amersham International). DNA–DNA hybridization experiments were carried

Fig. 2. Subset of a phylogenetic analysis (bootstrap topology is shown) of 64 16S rDNA sequences within the *Pseudomonas* genus. Percentages (500 bootstrap replications) indicate branches identified also by the two other methods (most parsimonious tree and branches positive at $P < 0.01$ for maximum-likelihood). The subset shown corresponds to the '*Pseudomonas syringae*-*Pseudomonas chlororaphis*-*Pseudomonas fluorescens*' groups of Anzai *et al.* (2000).

out using labelled DNA from strain CFBP 5737^T, at a reassociation temperature of 70 °C. Hybridization was conducted with the S₁ nuclease-trichloroacetic acid method as described by Crosa *et al.* (1973) and Grimont *et al.* (1980). The strains used for DNA-DNA hybridization are listed in Table 4. The T_m was determined when the percentage of DNA-DNA hybridization was between 50 and 66 % (Crosa *et al.*, 1973).

Except for *Pseudomonas mandelii* CFBP 4844^T, all strains in phenon 2 showed 60–100 % (mean, 77.75 %; SD, 12.2 %) DNA relatedness with strain CFBP 5737^T. For the two lowest values (60 and 66 %), the ΔT_m values were 3.9 and 4.3 °C, respectively. *Pseudomonas mandelii* CFBP 4844^T showed a very low level of similarity (46 %) with CFBP 5737^T. The percentage hybridization of labelled DNA from CFBP 5737^T with all other strains ranged from 12 to 60 %. For the upper values, 60 and 50 %, the ΔT_m values were 5.5 and 10.8 °C, respectively.

DNA base composition

The G+C content of strain CFBP 5737^T was determined by the thermal denaturation temperature (Marmur & Doty, 1962) and was calculated using the equation of Owen & Lapage (1976). *Escherichia coli* strain K12 CIP 54-117 (DNA G+C content 50.6 mol %) was used as a control. The DNA G+C content of strain CFBP 5737^T was 58.4 mol %, falling within the range of G+C contents for the genus *Pseudomonas* (55.2–67.2 mol %) (De Vos *et al.*, 1989; Palleroni, 1984).

Taxonomic conclusions

Twenty-one strains isolated from soils were compared with 47 reference strains of *Pseudomonas*, including 28 type strains. Nine strains included in phenon 2 could be differentiated from the strains of the other phenons by several biochemical characters. Within phenon 2,

Table 4. Levels of DNA reassociation among *Pseudomonas* strains tested

Source of unlabelled DNA		Phenon*	Reassociation (%) with labelled DNA from strain CFBP 5737 [†]
Species	Strain		
<i>Pseudomonas brassicacearum</i>	CFBP 5593 ^T	1	32
<i>Pseudomonas thivervalensis</i>	CFBP 5754 ^T	1	32
<i>Pseudomonas fluorescens</i> bv. II	CFBP 5758	1	24
<i>Pseudomonas fluorescens</i> bv. II	CFBP 5759	1	25
<i>Pseudomonas corrugata</i>	CFBP 2431 ^T	1	22
<i>Pseudomonas marginalis</i>	CFBP 3300 ^T	1	23
<i>Pseudomonas lini</i>	CFBP 5732	2	84
<i>Pseudomonas lini</i>	CFBP 5733	2	60 (3·9)
<i>Pseudomonas lini</i>	CFBP 5734	2	72
<i>Pseudomonas lini</i>	CFBP 5738	2	81
<i>Pseudomonas lini</i>	CFBP 5737 ^T	2	100
<i>Pseudomonas lini</i>	CFBP 5735	2	66 (4·3)
<i>Pseudomonas mandelii</i>	CFBP 4844 ^T	2	46
<i>Pseudomonas lini</i>	CFBP 2129	2	77
<i>Pseudomonas lini</i>	CFBP 5736	2	82
<i>Pseudomonas fluorescens</i> bv. I	CFBP 2102	4	22
<i>Pseudomonas fluorescens</i> bv. I	CFBP 2123	4	50 (10·8)
<i>Pseudomonas putida</i> bv. A	CFBP 5739	5	34
<i>Pseudomonas putida</i> bv. A	CFBP 5740	5	39
<i>Pseudomonas putida</i>	CFBP 5039	5	NT
<i>Pseudomonas putida</i> bv. A	CFBP 5741	5	35
<i>Pseudomonas putida</i> bv. A	CFBP 5744	5	32
<i>Pseudomonas putida</i> bv. A	CFBP 5745	5	38
<i>Pseudomonas putida</i> bv. A	CFBP 5746	5	40
<i>Pseudomonas putida</i>	CFBP 5030	6	NT
<i>Pseudomonas jessenii</i>	CFBP 4842 ^T	6	NT
<i>Pseudomonas fluorescens</i> bv. VI	CFBP 5756	7	29
' <i>Pseudomonas pavonacea</i> '	CFBP 5038	7	NT
<i>Pseudomonas fluorescens</i> bv. VI	CFBP 11751	7	39
<i>Pseudomonas fluorescens</i> bv. VI	CFBP 2392	7	33
<i>Pseudomonas</i> sp.	CFBP 5753	8	39
<i>Pseudomonas aureofaciens</i>	CFBP 2133 ^T	9	31
<i>Pseudomonas chlororaphis</i>	CFBP 5760	9	25
<i>Pseudomonas fluorescens</i> bv. V	CFBP 2130	9	29
<i>Pseudomonas putida</i> bv. A	CFBP 5742	11	38
<i>Pseudomonas putida</i> bv. A	CFBP 5743	11	34
<i>Pseudomonas</i> sp.	CFBP 5747	11	40
<i>Pseudomonas fluorescens</i> bv. II	CFBP 2125	IS	26
<i>Pseudomonas putida</i> bv. A	CFBP 2066 ^T	IS	14
<i>Pseudomonas aeruginosa</i>	CFBP 2466 ^T	IS	12
<i>Pseudomonas fluorescens</i> bv. II	CFBP 5757	IS	60 (5·5)

* IS, Isolated strains.

[†] Values in parentheses are values of ΔT_m (°C); NT, not tested.

Pseudomonas mandelii CFBP 4844^T could be discriminated from the eight other strains by four characters. Similarly, *Pseudomonas mandelii* CFBP 4844^T showed a very low DNA similarity value with CFBP 5737^T, whereas the eight other strains constitute a discrete DNA hybridization group: 60–100 % of DNA relatedness and ΔT_m less than 5 °C for the lowest DNA reassociation values (< 70 %). In this way the eight strains of phenon 2 constitute a homogeneous DNA

hybridization group. Among these eight strains, CFBP 2129 was previously classified as *Pseudomonas fluorescens*. Our data, based on a polyphasic approach, allowed us to conclude that the eight strains constitute a homogeneous group corresponding to a novel species.

The eight strains belong to two different and original siderovars. Studies performed so far on fluorescent

Pseudomonas spp. have indicated a good correlation between siderovar and species, i.e. one siderovar corresponds to one species (Achouak *et al.*, 2000; Meyer, 2000). However, for some species (*Pseudomonas aeruginosa*, *Pseudomonas tolaasii*) PVDs showed heterogeneity and strains were then differentiated into different siderovars (Meyer *et al.*, 1997; Munsch *et al.*, 2000). In the present study, siderotyping discrimination of the eight strains of phenon 2 reached the subspecies level and two siderovars were identified. *Pseudomonas mandelii* CFBP 4844^T demonstrated unique features at the level of its PVD system and was easily distinguishable from the eight other isolates of phenon 2.

For phylogenetic analysis, the 16S rDNA sequence of strain CFBP 5737^T was aligned and compared with the sequence of other *Pseudomonas* species. 16S rDNA sequence analysis showed that *Pseudomonas lini* does not belong to any recognized *Pseudomonas* species.

On the basis of the results of the polyphasic approach (phenotypic properties, DNA–DNA reassociation values, siderotyping and 16S rDNA gene sequencing), eight of the strains clustered in phenon 2 should be considered as a new *Pseudomonas* species. The name *Pseudomonas lini* sp. nov. is proposed with strain CFBP 5737^T as the type strain. Furthermore, strain ATCC 17513, formerly classified as a *Pseudomonas fluorescens* strain, is proposed to be now classified as a *Pseudomonas lini* strain.

Description of *Pseudomonas lini* sp. nov.

Pseudomonas lini (li'ni. L. n. *linum* flax; L. gen. n. *lini* of flax, referring to the plant genus, *Linum*, from which the type strain CFBP 5737^T was isolated).

Cells from stationary-phase culture of CFBP 5737^T are straight rods (0.5–1.0 by 1.5–5.0 µm), Gram-negative, motile by several polar flagella. Growth occurs between 4 and 36 °C, but not at 41 °C. The white-yellowish colonies on solid KB are smooth, circular with regular margins and moderately convex, and, after 2 days, produce a yellow-green fluorescence with UV light (365 nm). Aerobic growth, oxidase-positive, arginine dihydrolase-positive, not pectinolytic. Produces no hypersensitive reaction on tobacco leaves. The species reduces nitrogen oxides under anaerobic conditions. Hydrolyses gelatin, but not DNA and aesculin. Hydrolysis of Tween 80 is variable. Acid produced from mannitol, sorbitol and sucrose, but not from erythritol. Results obtained with Biotype 100 strips indicate that the strains are able to assimilate the following substrates: *cis*-aconitate, *trans*-aconitate, D-alanine, L-alanine, DL- α -amino-n-butyrate, L-arabinose, L-aspartate, betaine, caprate, caprylate, citrate, diaminobutane, β -D-fructose, fumarate, L-glutamate, glutarate, DL-glycerate, L-histidine, p-hydroxybenzoate, DL- β -hydroxybutyrate, D-galactose, D-gluconate, α -D-glucose, glycerol, DL-lactate, L-malate, D-mannitol, D-mannose, mucate, *myo*-inositol, L-proline, pro-

tocatechuate, quinate, D-saccharate, L-serine, D-sorbitol, succinate, sucrose and D-trehalose. The following substrates are not utilized as sole carbon and energy sources: adonitol, L-arabitol, D-cellobiose, *m*-coumarate, dulcitol, erythritol, aesculin, α -L-fucose, β -gentiobiose, gentisate, *m*-hydroxybenzoate, hydroxyquinoline- β -glucuronide, histamine, itaconate, α -lactose, lactulose, D-lyxose, maltose, maltotriose, D-melezitose, α -D-melibiose, *meso*-tartrate, 1-*O*-methyl- α -galactopyranoside, 1-*O*-methyl- β -galactopyranoside, maltitol, 5-keto-D-gluconate, 3-*O*-methyl-D-glucopyranose, 1-*O*-methyl- α -D-glucopyranoside, 1-*O*-methyl- β -D-glucopyranoside, palatinose, phenylacetate, 3-phenylpropionate, D-raffinose, α -L-rhamnose, L-sorbose, D-tagatose, tricarballoylate, trigonelline, tryptamine, D-turanose, D-xylose and xylitol. Assimilation of *N*-acetyl-D-glucosamine, DL- α -amino-n-valerate, D-arabitol, benzoate, ethanolamine, D-galacturonate, 2-keto-D-gluconate, D-glucosamine, D-glucuronate, α -ketoglutarate, D-malate, malonate, propionate, D-ribose, D-tartrate, L-tartrate, L-tryptophan and L-tyrosine is variable. Type strain is strain DLE411J^T which has been deposited in the Collection Française de Bactéries Phytopathogènes (CFBP, UMR de Pathologie Végétale, INRA-INH/Université, 49071 Beaucozéd cedex, France) under the number CFBP 5737^T and in the International Collection of Microorganisms from Plants (ICMP, Auckland, New Zealand) under the number ICMP 14138^T.

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