Full Paper

Recharacterization of *Pseudomonas fulva* Iizuka and Komagata 1963, and proposals of *Pseudomonas parafulva* sp. nov. and *Pseudomonas cremoricolorata* sp. nov.

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Seven *Pseudomonas fulva* strains obtained from culture collections were taxonomically studied. The seven strains were separated into three clusters (Clusters I to III) on the basis of 16S rRNA gene sequences, and located phylogenetically in the genus *Pseudomonas* sensu stricto. Further, the strains were classified into 4 groups (Groups I to IV) on the basis of DNA-DNA similarity. As a result, Cluster I was split into Groups I and II. Group I included the type strain of *P. fulva* and two strains, and levels of DNA-DNA similarity ranged from 88 to 100% among the strains. Group II contained two strains, and the level between the two strains ranged from 91 to 100%. Group III consisted of one strain. Group IV included one strain, and this strain showed a high level of DNA-DNA similarity with the type strain of *Pseudomonas straminea* NRIC 0164^T. Clusters II and III corresponded to Groups III and IV, respectively. The four groups were separated from one another and from related *Pseudomonas* species at the level from 3 to 45% of DNA-DNA similarity. The strains of Groups I, II, and III had ubiquinone 9 as the major quinone. According to numerical analysis by the use of 133 phenotypic characteristics, the seven *P. fulva* strains were split into four phenons (Phenons I to IV). The groups by DNA-DNA similarity corresponded well with the phenons produced by numerical taxonomy, and differential characteristics were recognized. Consequently, Group I was regarded as *P. fulva* because the type strain (NRIC 0180^T) of this species was included in this group. Strains in Group II were identified as a new species, *Pseudomonas parafulva* sp. nov., and the type strain is AJ 2129 (=IFO 16636=JCM 11244=NRIC 0501). NRIC 0181 in Group III was identified as a new species, *Pseudomonas cremoricolorata* sp. nov., and the type strain is NRIC 0181 (=IFO 16634=JCM 11246). NRIC 0182 in Group IV was identified as *P. straminea* on the basis of the high level of DNA-DNA similarity with the type strain of this species.

Key Words—*Pseudomonas*; *Pseudomonas cremoricolorata*; *Pseudomonas fulva*; *Pseudomonas parafulva*; *Pseudomonas straminea*

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Introduction

During the taxonomic study of *Pseudomonas* strains isolated from rice (Iizuka and Komagata, 1963a, b, c), and petroleum fields and oil-brine in Japan (Iizuka and Komagata, 1964a, b), including the type strains and

reference strains of this genus, Iizuka and Komagata (1963d) classified the genus *Pseudomonas* into three groups on the basis of pigmentation and isolation sources, namely, the fluorescent group, the achromogenic group, and the chromogenic group. The fluorescent group included strains that produced a water-soluble fluorescent pigment, the achromogenic group did not contain any pigment-producing strains, and the chromogenic group included strains that produced a water-insoluble yellow pigment. In addition, Iizuka and Komagata (1963c) isolated a water-soluble pigment and a water-insoluble yellow pigment producing strains from rice, included them into the fluorescent group, and named two species *Pseudomonas fulva* and *Pseudomonas straminea*. Recently, *P. straminea* was recharacterized (Uchino et al., 2000).

P. fulva was reported to have C_{16:0}, C_{16:1}, and C_{18:1} as the major cellular fatty acids (Ikemoto et al., 1978; Oyaizu and Komagata, 1983), and Q-9 as the major quinone (Oyaizu and Komagata, 1983; Yamada et al., 1982). On the basis of the 16S rRNA sequence, *P. fulva* belonged to the genus *Pseudomonas* sensu stricto (Anzai et al., 2000). Further, the relationships between three *P. fulva* strains and related species were reported on the basis of DNA-DNA similarity (Uchino et al., 2000).

In addition, Kodama et al. (1985) described *Pseudomonas oryzae* isolated from rice paddy and clinical specimens and *Pseudomonas luteola* from clinical specimens, respectively. These species produced a water-insoluble yellow pigment. Hildebrand et al. (1994) isolated a water-soluble fluorescent pigment and a water-insoluble yellow pigment producing *Pseudomonas* strains from walnut blight canker and named them *Pseudomonas flavescens*. Recently, Behrendt et al. (1999) reported the isolation of yellow-pigmented bacteria from the phyllosphere of grasses, and identified them as *Pseudomonas graminis*. Therefore, *P. fulva* might be related to *P. straminea*, *P. oryzae*, *P. flavescens*, and *P. graminis* in light of ecology because all above species are plant inhabiting and produce a water-insoluble yellow pigment.

This paper deals with the recharacterization of seven *P. fulva* strains obtained from culture collections, and the comparison with the strains of related species in light of phylogenetic relationships based on the 16S rRNA gene sequence, DNA-DNA similarity, and phenotypic characteristics. Further, *Pseudomonas parafulva* sp. nov. is proposed for the Group II based on

DNA-DNA similarity and the type strain is AJ 2129^T (=IFO 16636=JCM 11244=NRIC 0501), and *Pseudomonas cremoricolorata* sp. nov. is proposed for Group III, and the type strain is NRIC 0181^T (=IFO 16634=JCM 12246).

Materials and Methods

Bacterial strains. Seven *P. fulva* strains were obtained from culture collections, and subjected to this study. Strain designations and their sources are shown in Table 1.

Phenotypic characterization. Nutrient agar was used as a basal medium that contained 1% beef extract (Difco Laboratories, Detroit, MI, USA), 1% Bacto-Peptone (Difco), 0.5% NaCl, and 1.5% agar (pH 7.2). Unless otherwise stated, strains tested were cultivated at 30°C. Cell shape, Gram reaction, motility, and flagellar morphology were investigated in cells cultivated for 24 h. Gram staining was carried out by the method of Hucker-Conn modification (Hucker and Conn, 1923). Motility was investigated in the cells cultivated on nutrient agar at 25°C for 1 day by the hanging drop method. Flagellar morphology was determined by the staining method of Toda (1928). The accumulation of poly-β-hydroxybutyrate and the levan production were examined by the methods of Stanier et al. (1966). Production of a water-insoluble yellow pigment was investigated on nutrient agar and Tryptic Soy Agar (Difco) after 3 days of cultivation. Production of a fluorescent pigment was tested on *Pseudomonas* agar F (Difco), King B medium (King et al., 1954), and P-1 agar of Kato and Ito (Kato and Ito, 1983). Pyocyanin production was detected with *Pseudomonas* agar P (Difco) and King A medium (King et al., 1954). Growth on glutamate agar, reduction of nitrate to nitrite, nitrate respiration, and hydrolysis of starch were tested by the methods of Iizuka and Komagata (1963d). Hydrolysis of casein was determined by the production of a clear zone around a colony on nutrient agar containing 12% of skim milk after 7 days of cultivation at 25°C (Uchino et al., 2000). Arginine dihydrolase was detected by the method of Thornley (1960). The cleavage of protocatechuate was examined by the method of Hugh and Gilardi (1974). The oxidation-fermentation (O-F) test was performed by the method of Hugh and Leifson (1953). The catalase activity was detected by the production of bubbles by adding 3% hydrogen peroxide solution to resting cells. The oxidase test was performed with the

Table 1. Bacterial strains studied.

P. fulva

NRIC 0180^T←IAM 1529←H. Iizuka and K. Komagata, Y-8 (Iizuka and Komagata, 1963c)
 AJ 2126 (Source unknown)
 AJ 2129←CB-1 (Iizuka and Komagata, 1963c)
 AJ 2130←CP-9 (Iizuka and Komagata, 1963c)
 AJ 2131←CB-14 (Iizuka and Komagata, 1963c)
 NRIC 0181←IAM 1541←KS 0031←AJ 2127←H. Iizuka and K. Komagata, CC-8 (Iizuka and Komagata, 1963c)
 NRIC 0182←IAM 1587←KS 0029←H. Iizuka and K. Komagata, CB-10 (Iizuka and Komagata, 1963c)

P. aeruginosa

NRIC 0201^T←IFO 12689←AJ 2116←ATCC 10145←F. Kabanagh←Merck Sharp and Dohme Res. Lab. (=NCTC 10332=CCEB 481)

P. flavescens

B62^T, Univ. of California

P. fluorescens

IFO 14160^T←Kansai Med. Univ. (E. Yabuuchi; KM 2590)←R. Hugh, RH 818 (=ATCC 13525=NCTC 10038)

P. luteola

NRIC 0276^T←IAM 13000←K. Kodama, IAM, KS 0921←G. L. Gilardi, 4239 (CDC Group Ve-1) (=ATCC 43273=JCM 3352)

P. oryzae

NRIC 0277^T←IAM 1568←Replacement from S. Ikemoto et al., IAM, KS 0036 (=AJ 2197=ATCC 43272=JCM 2952=LMG 7040=KS 0036)

P. putida

IFO 14164^T←Kansai Med. Univ. (E. Yabuuchi; KM 1081)←J. Y. Homma←J. M. Shewan←NCIB 9494 (=ATCC 12633)

P. straminea

NRIC 0164^T←JCM 2783←KS 0028←AJ 2124←IAM 1598←H. Iizuka and K. Komagata, CB-7

Abbreviations: AJ, Central Research Laboratories, Ajinomoto Co., Ltd., Kawasaki, Japan; ATCC, American Type Culture Collection, Manassas, VA, USA; CCEB, Culture Collection of Entomogenous Bacteria, Institute of Entomology, Prague, Czech; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; IAM, IAM Culture Collection, Institute of Molecular and Cellular Bioscience, The University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Saitama, Japan; KM, strain designations used in this study; KS, Ikemoto et al., 1978; LMG, BCCMTM/LMG Bacteria Collection Laboratorium Microbiologie, Universiteit Gent, Gent, Belgium; NCIB, National Collection of Industrial and Marine Bacteria, Ltd., Aberdeen, Scotland, UK; NCTC, National Collection of Type Cultures, Central Public Health Laboratory Service, London, UK; NRIC, NODAI Culture Collection, Tokyo University of Agriculture, Tokyo, Japan.

cytochrome oxidase test paper (Eiken Chemical Co., Ltd., Tokyo, Japan). The assimilation of organic compounds was tested by the method of Stanier et al. (1966), and recorded after 3, 5, 7, and 14 days. Organic compounds used for the assimilation are shown in Table 5. Growth at pH 3.6 was investigated after 7 days of cultivation by the use of nutrient broth. Growth at 25, 30, 37, and 41°C was determined after 7 days of cultivation by the use of nutrient broth, and growth at 4°C was determined after 14 days.

Numerical analysis based on phenotypic characteristics. The phenotypic similarity was determined by the simple matching coefficient (S_{SM}) on the basis of 133 characteristics including 15 biochemical and physiological tests and the data of assimilation of 118 com-

pounds. Clustering was based on the unweighted pair group method with arithmetic average (Sneath and Sokal, 1973). Computation was carried out by using the NTSYS-pc ver. 2.00 program of Rohlf (1997).

Isolation of DNA and determination of DNA base composition. Cells were cultivated in nutrient broth for 24 h with shaking, and the cells were collected by centrifugation. DNA was extracted by the method of Saito and Miura (1963). DNA base composition was determined by HPLC as described by Tamaoka and Komagata (1984). Shodex ODS pack F-411 (Showa Denko K. K., Tokyo, Japan) was used.

DNA-DNA similarity. DNA-DNA hybridization was carried out by the method of Ezaki et al. (1989).

Quinone systems. Quinones were extracted with

ether-ethanol (3:1) and purified by thin layer chromatography (TLC). The quinones were determined by HPLC (Komagata and Suzuki, 1987). A column of Nova-Pak C18 (Waters Corp., Milford, MA, USA) was used.

Fatty acid composition. Cellular fatty acids were methylated with 5% HCl-methanol (Ikemoto et al., 1978; Komagata and Suzuki, 1987). The methylated esters were analyzed by gas chromatography by using a model GC-9A chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a capillary column DB-23 (0.25 mm by 60 m; Agilent Technologies Inc., Palo Alto, NY, USA) and a hydrogen flame ionization detector. Further, the methylated esters were separated for determination of 2- and 3-hydroxy fatty acids by TLC (Oyaizu and Komagata, 1983).

16S rRNA gene sequencing. Cloning and sequencing of 16S rRNA gene were carried out as described by Shida et al. (1997). Amplified 16S rRNA gene fragments were purified with a QIA Quick Spin PCR purification kit (Qiagen GmbH, Hilden, Germany), and used for sequencing templates. Sequencing was carried out by the method described by Sanger et al. (1977) with a Dye Terminator Cycle-sequencing FS Ready Reaction kit (Perkin-Elmer, Inc., Wellesley, CA, USA) and a model ABI 373A automatic DNA sequencer (Perkin-Elmer). Seven oligonucleotides 5'-CTGCTGCCTCCCGTAG-3' (primer 341R), 5'-GTAT-TACCGCGGCTGCTG-3' (primer 518R), 5'-TACGGG-AGGCAGCAG-3' (primer 356F), 5'-GTGCCAGCAGC-CGCGG-3' (primer 530F), 5'-GATTAGATACCCTGG-TAG-3' (primer 803F), 5'-ACTCAAAGGAATTGACGG-3' (primer 926F) and 5'-GCAACGAGCGCAACCC-3' (primer 1111F) were used as primers for sequencing of 16S rRNA gene fragments.

Phylogenetic analysis. 16S rRNA gene sequences were compared as described by Shida et al. (1997). Previously published 16S rRNA gene sequences were obtained from the EMBL/GenBank/DDBJ databases. The CLUSTAL X version 1.8 program (Thompson et al., 1997) was used for multiple alignment of sequences, determination of nucleotides substitution rate (K_{nuc} ; Kimura, 1980), construction of a neighbor-joining phylogenetic tree (Saitou and Nei, 1987), and 1,000 times bootstrap analysis for evaluation of phylogenetic tree topology (Felsenstein, 1985). Alignment gaps and unidentified base positions were not taken into account.

Accession numbers of 16S rRNA gene sequences.

DDBJ accession numbers for the 16S rRNA gene sequences of *Pseudomonas fulva* strains are as follows: AB060136 (*P. fulva* NRIC 0180^T), AB060134 (*P. fulva* AJ 2131), AB060131 (*P. fulva* AJ 2126), AB060132 (*P. parafulva* AJ 2129^T), AB060133 (*P. parafulva* AJ 2130), AB060137 (*P. cremoricolorata* NRIC 0181^T), and AB060135 (*P. straminea* NRIC 0182), respectively.

Results

Phylogenetic analysis

The seven *P. fulva* strains produced three clusters (Clusters I to III) on the basis of 16S rRNA gene sequences (Fig. 1). Cluster I contained strains NRIC 0180^T, AJ 2126, AJ 2131, AJ 2129 and AJ 2130. Values of the sequence similarity were greater than 99.9% among the strains. Cluster II included only one strain, NRIC 0181. A value of sequence similarity of this strain was less than 89.5% to other *Pseudomonas* species. Cluster III contained NRIC 0182, *P. straminea*, and *P. flavescens*. Values of the sequence similarity among the strains were greater than 89.4%.

DNA-DNA similarity

The seven *P. fulva* strains were classified into four groups (Groups I to IV) on the basis of the level of DNA-DNA similarity (Table 2). Group I contained NRIC 0180^T, AJ 2126, and AJ 2131, Group II included AJ 2129 and AJ 2130, Group III contained NRIC 0181, and Group IV included NRIC 0182. Levels of DNA-DNA similarity were high among the strains in Groups I and II, respectively, ranging from 88 to 100%. Thus, Cluster I based on the 16S rRNA sequence resulted in being split into two groups. Strains in Groups I to III showed low levels (<45%) of similarity with the type strains or reference strains of related *Pseudomonas* species. The level of DNA-DNA similarity of Group IV showed a high value with the type strain of *P. straminea* NRIC 0164^T, and low values with the type and reference strains of related *Pseudomonas* species, ranging from 5–44% similarity.

DNA base composition

The DNA base composition of seven *P. fulva* strains tested ranged from 60.0 to 62.1 mol% (Table 2).

Quinone systems

The major quinone was ubiquinone 9 (Q-9) in mem-

Fig. 1. Phylogenetic relationships of *Pseudomonas fulva* and some related species based on 16S rRNA gene sequences.

The branching pattern was generated by the neighbor-joining method. Bar indicates 0.01 nucleotide substitution per site. The sequence data were cited from gene data bank.

bers of Groups I, II, and III, and accounted for more than 95.0% of the total quinones (Table 3).

Fatty acid compositions

Major fatty acids of Groups I, II, and III were $C_{16:0}$ ($31.2 \pm 3.7\%$), $C_{16:1}$ ($25.6 \pm 5.4\%$), and $C_{18:1}$ ($25.5 \pm 14.9\%$). In addition, small amounts of 3-hydroxy $C_{10:0}$ and $C_{12:0}$ fatty acids and 2-hydroxy $C_{12:0}$ fatty acid were found, while 3-hydroxy $C_{14:0}$ and $C_{16:0}$ fatty acids were not detected (Table 4).

Phenotypic characteristics

All the strains studied were Gram negative and rod-shaped. Cells measured 1.0 by 2.0 μm in size, and were motile with polar flagella. The strains produced a water-insoluble yellow pigment on nutrient agar and Tryptic Soy Agar (Difco) but did not produce a water-

soluble fluorescent pigment or pyocyanin on any media tested. The strains tested were positive for growth on glutamate agar, catalase activity, oxidase activity, hydrolysis of casein, growth at 30°C, and the assimilation of D-glucose, D-fructose, D-gluconate, succinate, fumarate, L-lactate, citrate, and pyruvate. O-F test was oxidative. The strains were variable for arginine dihydrolase, growth at 37°C, and the assimilation of D-ribose, D-mannose, adonitol, 2-keto-D-gluconate, butyrate, valerate, caproate, isovalerate, itaconate, citraconate, glycerate, levulinate, Tween 80, *p*-hydroxybenzoate, inosine, glycine, L-ornithine, L-citrulline, and nicotinate. The strains tested were negative for starch hydrolysis, growth at 41°C, growth at pH 3.6, and the assimilation of cellobiose, lactose, soluble starch, inulin, PHB, phenol, salicylate, gentisate, xylenes, *p*-aminobenzoate, naphthalene, testosterone, D-

Table 2. DNA base compositions and levels of DNA-DNA similarity among *P. fulva* strains and strains of related species.

Strain No.	GC mol%	NRIC 0180 ^T	AJ 2129	NRIC 0181	NRIC 0164 ^T	IFO 14160 ^T	IFO 14164 ^T	B62	NRIC 0277 ^T	NRIC 0276 ^T
Group I										
<i>P. fulva</i>										
NRIC 0180 ^T	60.0	100	34	24	34	32	44	30	10	13
AJ 2126	61.5	96	36	39	34	39	40	16	13	10
AJ 2131	61.0	88	36	31	35	32	41	24	11	12
Group II										
<i>P. parafulva</i>										
AJ 2129 ^T	60.0	43	100	32	34	36	40	20	11	3
AJ 2130	60.0	45	91	36	34	35	41	20	9	3
Group III										
<i>P. cremoricolorata</i>										
NRIC 0181 ^T	62.1	39	34	100	32	39	35	34	13	5
Group IV										
<i>P. straminea</i>										
NRIC 0182	62.1	40	19	28	67	33	30	44	18	5
<i>P. straminea</i>										
NRIC 0164 ^T	62.5	40	15	30	100	29	19	45	20	5
<i>P. fluorescens</i>										
IFO 14160 ^T	60.5	9	11	19	19	100	22	22	11	7
<i>P. putida</i>										
IFO 14164 ^T	62.0	34	31	30	33	20	100	27	8	8
<i>P. flavescens</i>										
B62	62.8	5	13	19	37	9	17	100	14	7
<i>P. oryzae</i>										
NRIC 0277 ^T	66.4	16	10	22	35	16	16	16	100	9
<i>P. luteola</i>										
NRIC 0276 ^T	54.8	5	5	3	10	4	5	7	9	100

Table 3. Ubiquinone systems of strains of *Pseudomonas fulva* and related species.

Species	Strain No.	Ubiquinone (%)		
		Q-8	Q-9	Q-10
<i>P. fulva</i>	NRIC 0180 ^T	3.1	95.0	2.0
	AJ 2126	—	100.0	—
	AJ 2131	2.7	97.4	—
<i>P. parafulva</i>	AJ 2129	3.6	95.7	0.7
<i>P. cremoricolorata</i>	NRIC 0181 ^T	3.8	95.4	0.8
<i>P. straminea</i>	NRIC 0164 ^T	1.8	97.4	0.8
<i>P. fluorescens</i>	IFO 14160 ^T	8.5	91.5	—
<i>P. putida</i>	IFO 14164 ^T	2.5	97.6	—
<i>P. flavescens</i>	B62 ^T	—	95.7	4.3
<i>P. oryzae</i>	NRIC 0277 ^T	—	100.0	—
<i>P. luteola</i>	NRIC 0276 ^T	—	100.0	—
<i>P. aeruginosa</i>	NRIC 0201 ^T	—	100.0	—

aspartate, D-tryptophane, and glutathione. The *P. fulva* strains showed the ortho cleavage of protocatechuate, but NRIC 0181 did not decompose *p*-hydroxybenzoate. Other data are shown in Table 5.

Numerical analysis based on phenotypic characteristics

A total of 133 phenotypic characteristics were subjected to numerical analysis. Four phenons (Phenons I to IV) were constructed from the seven strains at 70% S_{SM} (Fig. 2). Phenon I contained NRIC 0180^T, AJ 2131, and AJ 2126, Phenon II included AJ 2129 and AJ 2130, and Phenon III consisted only of NRIC 0181. Phenons I, II and III joined together in one phenotypic cluster at 82% S_{SM} . Phenon IV included only NRIC 0182, and joined in the phenon of *P. straminea* at 90% S_{SM} , and in the phenon including NRIC 0182, *P. straminea* NRIC 0167^T, and *P. flavescens* B62^T at 82% S_{SM} .

Table 4. The cellular fatty acid compositions of strains of *Pseudomonas fulva* and related species.

Strain		Fatty acid composition (%) ^a													
		Straight-chain acid									3-Hydroxy acid		12:0 2-Hydroxy acid	17:0 Cyclo- propane acid	Unknown peak
		12:0	13:0	14:0	15:0	16:0	16:1	18:0	18:1	19:0	10:0	12:0			
<i>P. fulva</i>	NRIC 0180 ^T	1.1		0.5	T	33.6	29.2	1.0	27.6		0.9	1.5	4.5		T
	AJ 2126	2.7		T	T	27.5	22.1	1.1	40.4		1.2	1.5	3.3		T
	AJ 2131	T		T	T	32.9	25.9	1.1	31.3		1.5	0.9	5.4		
<i>P. parafulva</i>	AJ 2129 ^T	8.2		3.6		34.8	20.2		10.6		8.2	5.2	9.1		
<i>P. cremoricolorata</i>	NRIC 0181 ^T	9.1		1.5		33.3	30.9	0.9	11.3		5.8	3.7	3.4		
<i>P. straminea</i>	NRIC 0164 ^T	4.6		0.5		20.7	20.3	1.2	49.2		1.1	0.9	1.2		T
<i>P. fluorescens</i>	IFO 14160 ^T	1.3		T		31.8	29.9	1.6	26.8		2.4	1.0	4.9		
<i>P. putida</i>	IFO 14164 ^T	1.5		0.6	T	39.9	19.1	2.0	22.7		2.9	3.1	8.0		T
<i>P. flavescens</i>	B62 ^T	7.2		0.7	T	22.6	19.7	1.3	45.4		1.5	1.0	T		
<i>P. oryzihabitans</i>	NRIC 0277 ^T	2.6		T		24.3	16.5	1.1	48.6		1.3	1.4	3.8		T
<i>P. luteola</i>	NRIC 0276 ^T	4.9		T		21.9	16.7	0.9	47.2		2.3	2.2	3.7		
<i>P. aeruginosa</i>	NRIC 0201 ^T	5.8		0.9		20.9	19.5	T	45.6		2.1	2.4	2.6		T

^a Numbers refer to the ratio of the fatty acid to a total of acids. T, acid present less than 0.5%.

Discussion

Phylogenetic analysis based on the 16S rRNA gene sequence warrants the seven *P. fulva* strains studied to be included in the genus *Pseudomonas*. Further, the *P. fulva* strains were separated into four groups (Groups I to IV) based on DNA-DNA similarity. The groups by DNA-DNA similarity corresponded well with the phenons produced by numerical analysis. DNA base composition of all strains tested fell into the range of genus *Pseudomonas*, 58–70 mol% (Palleroni, 1984). The major ubiquinone and major fatty acids fell into the category of the genus *Pseudomonas* (Oyaizu and Komagata, 1983). Groups I, II and III were differentiated from one another by arginine dihydrolase, growth at 37°C, and the assimilation of D-ribose, D-mannose, adonitol, 2-keto-D-gluconate, butyrate, valerate, caprate, isovalerate, itaconate, citraconate, glycerate, levulinate, Tween 80, *p*-hydroxybenzoate, inosine, glycine, L-ornithine, L-citrulline, and nicotinate.

Group I contained NRIC 0180^T, AJ 2126, and AJ 2131, and these strains were identified as *P. fulva* because the type strain of this species was included in this group. In the previous paper (Uchino et al., 2000), *P. fulva* KM 310 and AJ 2126 were described to originate from CB-10, but this citation was not correct. The source of KM 310 and AJ 2126 should be described

unknown because there is no record evidenced their origin. Group II included AJ 2129 and AJ 2130, and identified as a new species, *Pseudomonas pseudo-fulva* nov. sp. The type strain of this species is AJ 2129^T. Group III contained NRIC 0181, and identified as a new species, *Pseudomonas cremoricolorata* sp. nov. The type strain is NRIC 0181^T. While, NRIC 0182 in the Group IV was identified as *P. straminea* on the basis of a high level of DNA-DNA similarity with the type strain of this species. *P. straminea* was separated from *P. flavescens* by the level of DNA-DNA similarity (Uchino et al., 2000). *P. fulva*, *P. parafulva*, *P. cremoricolorata*, and *P. straminea* were differentiated from related *Pseudomonas* species on the basis of DNA-DNA similarity, and differential phenotypic characteristics are shown in Table 6.

According to the study of Iizuka and Komagata (1963c, d), *P. fulva* and *P. straminea* belonged to the fluorescent group because they produced both a water-insoluble yellow pigment and a water-soluble greenish yellow pigment. In contrast to the original description, the *P. fulva* strains studied did not produce a water-soluble fluorescent pigment. *P. flavescens* was reported to produce both a yellow cellular pigment and a fluorescent pigment. *P. fulva* strains that produce both pigments probably encountered in fresh isolates. *P. fulva* KM 307 (the type strain) was reported to pro-

[illegible]

[illegible]

Table 5. (Continued)

Phenotypical characteristics	Phenon I <i>P. fulva</i> (3)	Phenon II <i>P. parafulva</i> (2)	Phenon III <i>P. cremoricolorata</i> (1)	Phenon IV <i>P. straminea</i> (1)	<i>P. straminea</i> NRIC 0164	<i>P. aeruginosa</i> NRIC 0201	<i>P. flavescens</i> B62	<i>P. fluorescens</i> IFO 14160	<i>P. luteola</i> NRIC 0276	<i>P. oryzae</i> NRIC 0277	<i>P. putida</i> IFO 14164
L(+)-Tartrate	—	—	—	—	—	—	—	—	—	+	+
<i>m</i> -Tartrate	d(1/3)	—	—	+	—	—	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	+	+	+
D-Galactonolactone	d(1/3)	—	—	+	+	—	+	+	+	+	—
Pyruvate	+	+	+	+	+	+	+	+	+	+	+
Levulinate	+	—	—	—	—	+	+	—	—	—	+
DL-2-Aminobutyrate	—	—	—	—	—	—	—	—	—	—	+
PHB	—	—	—	—	—	—	—	—	—	—	—
Tween 80	+	—	+	—	—	—	—	—	+	+	—
Ethanol	+	+	+	—	—	+	—	—	+	+	+
<i>n</i> -Propanol	+	+	+	—	—	+	—	—	+	+	+
<i>n</i> -Butanol	—	—	—	—	—	+	—	—	+	—	+
Isobutanol	d(1/3)	+	—	—	—	+	—	—	+	+	—
1-Hexanol	d(2/3)	+	—	—	—	—	—	—	+	+	+
Geraniol	—	—	—	—	—	+	—	—	—	—	—
Ethylene glycol	—	—	—	—	—	—	—	—	—	—	—
Propylene glycol	+	+	+	—	—	+	—	—	+	—	+
Phenol	—	—	—	—	—	—	—	—	—	—	—
Benzoate	—	—	—	—	+	+	—	—	—	—	+
Salicylate	—	—	—	—	—	—	—	—	—	—	—
<i>m</i> -Hydroxybenzoate	—	—	—	—	—	—	+	+	—	—	+
<i>p</i> -Hydroxybenzoate	—	+	—	+	+	+	+	+	+	+	+
Protocatechuate	—	—	—	—	—	—	—	—	+	—	—
Gentisate	—	—	—	—	—	—	—	—	—	—	—
D-Mandelate	—	—	—	—	—	—	—	—	—	—	+
L-Mandelate	—	—	—	—	—	+	—	—	—	—	+
Benzylformate	d(1/3)	—	—	+	—	+	—	—	—	—	+
Xylene	—	—	—	—	—	—	—	—	—	—	—
Phenyl acetate	d(2/3)	—	—	—	—	—	—	—	—	—	+
<i>p</i> -Aminobenzoate	—	—	—	—	—	—	—	—	—	—	—
Naphthalene	—	—	—	—	—	—	—	—	—	—	—
Inosine	+	+	—	—	—	+	+	+	+	+	+
Taurocholate	—	—	—	—	—	—	—	—	—	—	+
Testosterone	—	—	—	—	—	—	—	—	—	—	—
Glycine	+	+	—	—	—	+	—	—	—	—	+
β-Alanine	+	+	+	—	—	+	+	+	+	+	+
L-Valine	+	+	+	—	—	+	—	+	—	+	+
L-Leucine	+	+	+	—	—	+	+	+	+	+	+
L-Isoleucine	+	+	+	—	—	+	—	+	+	+	+
D-Aspartate	—	—	—	—	—	—	—	—	—	—	—
L-Serine	+	+	+	—	+	+	+	+	+	+	+
L-Threonine	d(2/3)	—	—	—	—	—	—	—	—	—	+
L-Phenylalanine	d(2/3)	+	—	—	—	+	—	+	—	—	+

Table 5. (Continued)

Phenotypical characteristics	Phenon I <i>P. fulva</i> (3)	Phenon II <i>P. parafulva</i> (2)	Phenon III <i>P. cremoricolorata</i> (1)	Phenon IV <i>P. straminea</i> (1)	<i>P. straminea</i> NRIC 0164	<i>P. aeruginosa</i> NRIC 0201	<i>P. flavescens</i> B62	<i>P. fluorescens</i> IFO 14160	<i>P. luteola</i> NRIC 0276	<i>P. oryzihabitans</i> NRIC 0277	<i>P. putida</i> IFO 14164
L-Tyrosine	d(1/3)	+	–	–	–	+	–	+	+	–	+
L-Tryptophan	–	–	–	–	–	+	–	+	–	–	–
D-Tryptophan	–	–	–	–	–	–	–	–	–	–	–
L-Lysine	+	+	+	–	–	+	–	–	+	–	+
L-Ornithine	+	+	–	–	–	+	–	+	+	–	+
L-Histidine	d(2/3)	d(1/2)	–	–	+	+	+	+	+	–	+
DL-Norvaline	–	–	–	–	–	+	–	–	–	–	+
Creatine	–	–	–	–	–	–	–	–	–	–	+
Anthranilate	–	–	–	–	–	+	–	–	–	–	–
L-Citrulline	+	–	–	–	–	–	+	–	–	–	+
Hippurate	–	–	–	–	–	–	–	–	–	–	+
Pantothenate	–	–	–	–	–	–	–	–	+	–	–
Nicotinate	+	–	–	–	+	–	–	+	–	–	+
Glutathione	–	–	–	–	–	–	–	–	–	–	–
Trigonelline	+	+	+	+	+	–	–	–	–	–	+
Acetamide	–	–	–	–	–	+	–	–	–	–	–
Butylamine	+	+	+	–	–	–	–	–	–	–	+
Tryptamine	–	d(1/2)	–	–	–	–	–	–	–	–	+
Amylamine	d(1/3)	+	+	–	–	–	+	–	–	–	+
Benzylamine	–	–	–	–	–	+	–	–	–	–	+
Betaine	+	+	+	–	+	+	+	+	+	+	+
Dodecane	d(1/3)	–	–	–	–	–	–	–	–	–	–

Characteristics are scored as: +, positive reaction; –, negative reaction; d, strain-dependent reaction.

Numbers in parentheses indicate the number of strains showing a positive reaction.

^a *P. cremoricolorata* did not decompose protocatechuate.

duce a water-insoluble yellow pigment with an absorption peak at 444.8 nm (Uchino et al., 2000).

On the basis of the production of an insoluble yellow pigment and a limited isolation source pertaining to plant materials, *P. fulva*, *P. pseudofulva*, *P. cremoricolorata*, *P. straminea*, *P. oryzihabitans*, *P. flavescens*, and *P. graminis* were considered to be related to one another in ecological point of view.

Descriptions

Pseudomonas parafulva sp. nov.

(*parafulva* pa. ra. ful'va; Gr. prefix *para* like; L. fem. adj. *fulvus*, yellow; N. L. fem. adj. *parafulva fulva*-like)

Cells are rods, measuring 0.6 to 0.8 by 1.4 to

1.6 µm, with rounded ends. Gram negative. The cells occur singly, rarely in pairs, and are motile with polar flagella. Endospores are not formed. Granules of poly-β-hydroxybutyrate do not accumulate in the cells. Colonies on nutrient agar are smooth, entire, flat to convex, and pale yellow. A water-insoluble yellow pigment is produced on nutrient agar and Tryptic Soy Agar (Difco). A water-soluble fluorescent pigment is not produced on *Pseudomonas* agar F, King B medium, or Kato and Ito's P-1 medium. No pyocyanin is produced on *Pseudomonas* agar P. Growth occurs on glutamate agar. Metabolism is strictly respiratory. Oxidative but not fermentative. Catalase and oxidase are produced. Nitrate is not reduced to nitrite in nitrate broth. Nitrate respiration is negative. Arginine dihydro-

Fig. 2. Dendrogram showing the phenotypic relationship among the strains studied, generated by using the simple matching coefficient and the unweighted pair group arithmetic average algorithm.

Table 6. Differential characteristics of *P. fulva*, *P. parafulva*, and *P. cremoricolorata*, and other related *Pseudomonas* species.

Phenotypical characteristics	<i>P. fulva</i> (3) ^a	<i>P. parafulva</i> (2)	<i>P. cremoricolorata</i> (1)	<i>P. straminea</i> NRIC 0164	<i>P. aeruginosa</i> NRIC 0201	<i>P. flavescens</i> B62	<i>P. fluorescens</i> IFO 14160	<i>P. luteola</i> NRIC 0276	<i>P. oryzae</i> NRIC 0277	<i>P. putida</i> IFO 14164
Arginine dihydrolase	+	+	—	—	+	—	+	+	—	+
Growth at 4°C	+	d(1/2)	+	+	—	+	+	—	—	+
Growth at 37°C	+	+	—	+	+	—	—	+	+	+
Assimilation of										
D-Ribose	+	+	—	+	+	—	+	+	+	+
D-Mannose	+	+	—	+	—	+	+	+	+	—
Adonitol	—	+	—	—	—	—	+	—	—	—
2-Keto-D-gluconate	+	+	—	—	+	—	—	+	+	+
Butyrate	—	+	—	—	+	+	+	+	—	+
Valerate	—	+	—	—	+	+	+	—	—	+
Caprate	+	+	—	+	+	+	+	+	+	+
Isovalerate	+	+	—	—	+	—	—	—	—	+
Itaconate	+	—	—	+	+	+	+	—	+	+
Citraconate	—	—	+	—	—	—	+	—	+	—
Glycerate	+	+	—	—	—	—	—	+	+	+
Levulinate	+	—	—	—	+	+	—	—	—	+
Tween 80	+	—	+	—	—	—	—	+	+	—
p-Hydroxy benzoate	—	+	—	+	+	+	+	+	+	+
Inosine	+	+	—	—	+	+	+	+	+	+
Glycine	+	+	—	—	+	—	—	—	—	+
L-Ornithine	+	+	—	—	+	—	+	+	—	+
L-Citrulline	+	—	—	—	—	+	—	—	—	+
Nicotinate	+	—	—	+	—	—	+	—	—	+

^a The number of strains studied and the accession numbers of culture collections.

lase is produced. Levan is not produced from sucrose. Starch and casein are not hydrolyzed. Growth occurs at 37°C but not at 41°C. Growth does not occur at pH 3.6. Cleavage of protocatechuate is of the ortho type. The assimilation pattern is shown in Table 5.

DNA base composition is 60 mol%.

The major fatty acids are $C_{16:0}$, $C_{16:1}$, and $C_{18:1}$, and major 3-hydroxy fatty acids are $C_{10:0}$ and $C_{12:0}$.

The major quinone is ubiquinone 9.

Distributed to rice paddy.

The type strain is AJ 2129^T (=IFO 16636=JCM 11244=NRIC 0501), of which DNA base composition is 60.0 mol%. This strain was isolated from Japanese rice paddy by Iizuka and Komagata in 1963 (Iizuka and Komagata, 1963c).

Pseudomonas cremoricolorata sp. nov.

(*cremoricolorata* cre.mo.ri.co.lo.ra'ta; L. mas. n. *cremor*, juice, cream; L. adj. *coloratus*, colored; N. L. fem. adj. cream-colored)

Cells are rods measuring 0.8 by 1.6 µm, with rounded ends. Gram negative. The cells occur singly, rarely in pairs, and are motile with polar flagella. Endospores are not formed. Granules of poly-β-hydroxybutyrate do not accumulate in the cells. Colonies on nutrient agar are smooth, entire, flat to convex, and creamy yellow. A water-insoluble yellow pigment is produced on nutrient agar and Tryptic Soy Agar (Difco). A water-soluble fluorescent pigment is not produced on *Pseudomonas* agar F, King B medium, or Kato and Ito's P-1 medium. Pyocyanin is not produced on *Pseudomonas* agar P. Growth occurs on glutamate agar. Metabolism is strictly respiratory. Oxidative but not fermentative. Catalase and oxidase are produced. Nitrate is not reduced to nitrite in nitrate broth. Nitrate respiration is negative. *p*-Hydroxybenzoate is not decomposed. Arginine dihydrolase is not produced. Levan is not produced from sucrose. Starch and casein are not hydrolyzed. Growth occurs at 4°C but not at 37°C. Growth does not occur at pH 3.6. The assimilation pattern is shown in Table 5.

DNA base composition is 62.1 mol%.

The major fatty acids are $C_{16:0}$, $C_{16:1}$, and $C_{18:1}$, and major 3-hydroxy fatty acids are $C_{10:0}$ and $C_{12:0}$.

The major ubiquinone is ubiquinone 9.

Distributed to rice paddy.

The type strain is NRIC 0181^T (=IFO 16634=JCM 11246), of which DNA base composition is 62.1 mol%. This strain was isolated from Japanese paddy by

Iizuka and Komagata in 1963 (Iizuka and Komagata, 1963c).

Recharacterization of *Pseudomonas fulva* Iizuka and Komagata 1963^{AL}

Cells are rods measuring 0.6 to 0.8 by 1.4 to 1.8 µm, with rounded ends. Gram negative. The cells occur singly, rarely in pairs, and are motile with polar flagella. Endospores are not formed. Granules of poly-β-hydroxybutyrate do not accumulate in the cells. Colonies on nutrient agar are smooth, entire, flat to convex, and pale brownish yellow. A water-insoluble yellow pigment is produced on nutrient agar and Tryptic Soy Agar (Difco). A water-soluble fluorescent pigment is not produced on *Pseudomonas* agar F, King B medium, or Kato and Ito's P-1 medium. No pyocyanin is produced on *Pseudomonas* agar P. Growth occurs on glutamate agar. Metabolism is strictly respiratory. Oxidative but not fermentative. Catalase and oxidase are produced. Nitrate is not reduced to nitrite. Nitrate respiration is negative. Arginine dihydrolase is produced. Levan is not produced from sucrose. Starch and casein are not hydrolyzed. Growth occurs at 4 and 37°C but not at 41°C. Growth does not occur at pH 3.6. Cleavage of protocatechuate is of the ortho type. The assimilation pattern is shown on Table 5.

DNA base composition ranges from 60.0 to 61.5 mol%.

The major fatty acids are $C_{16:0}$, $C_{16:1}$, and $C_{18:1}$, and major 3-hydroxy fatty acids are $C_{10:0}$ and $C_{12:0}$.

The major ubiquinone is ubiquinone 9.

Distributed to rice paddy.

The type strain is NRIC 0180^T (=IFO 16637=JCM 11242), of which DNA base composition is 60.0 mol%. This strain was isolated from Japanese rice paddy by Iizuka and Komagata in 1963 (Iizuka and Komagata, 1963c).

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