Pseudomonas flavescentis sp. nov., Isolated from Walnut Blight Cankers

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Two pseudomonad strains that produce a yellow cellular pigment, in addition to a diffusible fluorescent pigment on Kings medium B, were isolated from cankers on walnut trees. Biochemical properties, such as a positive oxidase reaction, a negative arginine dihydrolase reaction, and the production of a fluorescent pigment, in addition to the results of an extensive nutritional characterization study and DNA-DNA hybridization experiments, indicated that these strains belong to a new *Pseudomonas* rRNA group I species. This conclusion was supported by the results of a determination of the sequence of the PCR-amplified 16S rRNA gene and a comparison with the 16S rRNA genes of other bacterial species. The genomic DNAs of the strains had a base composition of 63 mol% G+C. The name *Pseudomonas flavescentis* sp. nov. is proposed. Strain B62 (= NCPPB 3063) is the type strain of the species.

During a study of *Xanthomonas campestris* pv. *juglandis* causing cankers on walnut trees, it was observed that some of the cankers were covered with a bright yellow bacterial mass oozing from the lesions. It was assumed that this material consisted primarily of *X. campestris* pv. *juglandis* cells, since similar phenomena are not uncommon with cankers caused by other bacterial plant pathogens. However, isolation of bacteria from the lesion revealed that the largest proportion of the bacteria were fluorescent pseudomonads that produce a yellow cellular pigment. *X. campestris* pv. *juglandis* was also present, although in smaller numbers. In this paper we describe the results of a taxonomic study of this canker-inhabiting pseudomonad and ascribe this organism to a new species, for which the name *Pseudomonas flavescentis* is proposed.

MATERIALS AND METHODS

Bacterial strains. Two pseudomonad strains were isolated 6 weeks apart from walnut trees in Orinda, Calif. Strain B62^T (T = type strain) was isolated from canker tissue, and strain B62-D5 was isolated from bacterial ooze on a canker. Other strains used for comparative purposes are listed in Table 1. YDCP medium (17) was used for isolation and maintenance of strains.

Nutritional and biochemical characteristics. Denitrification, poly- β -hydroxybutyrate accumulation, the oxidase reaction, the presence of arginine dihydrolase, and hydrolysis of gelatin, starch, and Tween 80 were examined by using methods described by Palleroni and Doudoroff (23). Rutin glycosidase activity was tested by using the basal medium of Ayers et al. (2) (1 g of ammonium sulfate per liter, 0.2 g of potassium chloride per liter, 0.2 g of magnesium sulfate per liter, 15 g of agar per liter; pH 7.2) supplemented with rutin and glucose as described elsewhere (5). β -Glucosidase activity, pectolytic activity, ice nucleation activity, the ability to cause soft rot of potatoes, the production of levan, and hypersensitivity were examined by the methods described previously (9). Carbon source utilization tests were conducted by adding carbon sources (0.1%, wt/vol)

to the mineral base media of Ayers et al. (2) and Palleroni and Doudoroff (23). Most of the carbon stock solutions were sterilized by autoclaving; the only exceptions were the solutions sensitive to heat, which were sterilized by filtration. Cultures were inoculated by placing a 0.03-ml drop of a turbid bacterial suspension on an agar medium. Growth was examined after 3, 7, 14, and 21 days of incubation at 28°C. Strains inoculated onto minimal medium plates without any added carbon source were used for comparison. Weak growth was scored as a negative result.

Flagellum characterization. Flagellation was observed by using the method of Hoeniger (10). Grids were shadowed with platinum. Flagella were examined with a Phillips model 200 transmission electron microscope.

Plant inoculation. Bacterial suspensions of the organisms being studied, alone or in mixtures with *X. campestris* pv. *juglandis*, were inoculated into immature leaves of walnut (*Juglans regia*) by using Carborundum (9). Shoot tips were also inoculated by means of needle pricks (9).

Pigment analysis. Yellow cellular pigment was observed on YDCP medium (17) and on King media A and B (15). In addition, on King medium B a diffusible fluorescent pigment which was visible under UV light was produced. The yellow cellular pigment was analyzed spectrophotometrically (11). Cells were grown on nutrient agar plates for 4 days and then were scraped off and placed in small beakers. Methanol was added, the mixture was stirred, anhydrous sodium sulfate was added to remove water, and the liquid phase was centrifuged. An absorption spectrum for the supernatant was obtained by using a Cary model 14 double-beam spectrophotometer. Preparations of pigments extracted from *Xanthomonas campestris* pv. *campestris* ATCC 33913^T, *X. campestris* pv. *incanae* ATCC 13462^T, *X. campestris* pv. *juglandis* ICPB 411^T, and *X. campestris* pv. *malvacearum* ATCC 9924 were used for comparison.

The diffusible fluorescent pigment was analyzed spectrophotometrically by using a Gilford Response II spectrophotometer (Ciba Corning, Oberlin, Ohio). Bacteria were inoculated into King medium B broth (15) and were grown in a shaking incubator at 250 rpm and 28°C for 48 h. One milliliter of each culture was centrifuged in an Eppendorf microcentrifuge at 12,000 $\times g$ for 2 min, and the absorption spectra of the supernatants were determined. Strains B62^T and B62-D5 were compared with other fluorescent pigment-producing pseudo-

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TABLE 1. Bacterial strains studied

Taxon	Strain ^a
<i>Alcaligenes denitrificans</i> subsp. <i>denitrificans</i>	ATCC 15173 ^T
<i>Alcaligenes eutrophus</i>	ATCC 17697 ^T
<i>Alcaligenes faecalis</i>	ATCC 8750 ^T
<i>Alcaligenes faecalis</i>	ATCC 15554 ^T
<i>Acidovorax delafieldii</i>	ATCC 17505 ^T
<i>Acidovorax facilis</i>	ATCC 11228 ^T
<i>Burkholderia cepacia</i>	ATCC 10856
<i>Burkholderia cepacia</i>	ATCC 35416 ^T
<i>Comamonas acidovorans</i>	ATCC 11299
<i>Comamonas testosteroni</i>	ATCC 11996 ^T
<i>Comamonas testosteroni</i>	ATCC 17510
<i>Hydrogenophaga palleronii</i>	ATCC 17724 ^T
<i>Pseudomonas aeruginosa</i>	ATCC 10145 ^T
<i>Pseudomonas agarici</i>	ATCC 25943
<i>Pseudomonas alcaligenes</i>	ATCC 14909 ^T
<i>Pseudomonas andropogonis</i>	ATCC 23060
<i>Pseudomonas avenae</i>	ATCC 19860 ^T
<i>Pseudomonas aureofaciens</i>	ATCC 17415
<i>Pseudomonas cichorii</i>	ATCC 10857 ^T
<i>Pseudomonas chlororaphis</i>	ATCC 9446 ^T
<i>Pseudomonas diminuta</i>	ATCC 11568 ^T
<i>Pseudomonas fluorescens</i>	ATCC 13525 ^T
<i>Pseudomonas huttiensis</i>	ATCC 14670 ^T
<i>Pseudomonas gladioli</i> pv. <i>gladioli</i>	ATCC 10248 ^T
<i>Pseudomonas mendocina</i>	ATCC 25411 ^T
<i>Pseudomonas putida</i>	ATCC 12633 ^T
<i>Pseudomonas pyrocinia</i>	ATCC 15958 ^T
<i>Pseudomonas saccharophila</i>	ATCC 15946 ^T
<i>Pseudomonas solanacearum</i>	ATCC 10692
<i>Pseudomonas synxantha</i>	ATCC 9890 ^T
<i>Pseudomonas syringae</i>	ATCC 19304 ^T
<i>Serratia marcescens</i>	ATCC 21074
<i>Xanthomonas campestris</i>	ATCC 19865
<i>Xanthomonas campestris</i> pv. <i>begoniae</i>	NCPPB 1926 ^T
<i>Xanthomonas campestris</i> pv. <i>campestris</i>	ATCC 33913 ^T
<i>Xanthomonas campestris</i> pv. <i>celebensis</i>	ATCC 19045 ^T
<i>Xanthomonas campestris</i> pv. <i>glycines</i>	ICPB XP175 ^T
<i>Xanthomonas campestris</i> pv. <i>incanae</i>	ATCC 13462 ^T
<i>Xanthomonas campestris</i> pv. <i>juglandis</i>	NCPPB 411 ^T
<i>Xanthomonas campestris</i> pv. <i>malvacearum</i>	ATCC 9924
<i>Xanthomonas campestris</i> pv. <i>oryzicola</i>	ICPB XO111 ^T
<i>Xanthomonas campestris</i> pv. <i>pelargonii</i>	ATCC 8721
<i>Xanthomonas campestris</i> pv. <i>phaseoli</i>	ATCC 9563 ^T
<i>Xanthomonas campestris</i> pv. <i>taraxaci</i>	ATCC 19318 ^T
<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>	ATCC 35937 ^T
<i>Xanthomonas campestris</i> pv. <i>vitians</i>	ATCC 19320 ^T
<i>Stenotrophomonas maltophilia</i>	ATCC 17806 ^T

^a ATCC, American Type Culture Collection, Rockville, Md.; ICPB, International Collection of Phytopathogenic Bacteria, Berkeley, Calif.; NCPPB, National Collection of Plant Pathogenic Bacteria, Hertfordshire, United Kingdom.

monads, including *Pseudomonas aeruginosa* ATCC 10145^T, *Pseudomonas fluorescens* ATCC 13525^T, *Pseudomonas putida* ATCC 12633^T, *Pseudomonas syringae* ATCC 19304^T, and *Pseudomonas cichorii* ATCC 10857^T. *Pseudomonas mendocina* ATCC 24511^T, which produced a yellow cellular pigment but not a fluorescent diffusible pigment, was also used for comparison.

DNA isolation and purification. DNA was isolated and purified by using the method of Marmur (20), as modified by Ballard et al. (3).

DNA base composition. The guanine-plus-cytosine (G+C) contents of DNAs were determined by thermal denaturation (19) of solutions containing 25 µg of DNA per ml in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0), using a Gilford Response II spectrophotometer equipped with

a thermoprogrammer. The G+C content was estimated by using the melting temperature and the formula of Mandel and Marmur (19). *Pseudomonas aureofaciens* ATCC 17415 and *P. putida* ATCC 12633^T genomic DNA preparations, which had G+C contents of 63.6 and 62.5 mol%, respectively (18), were used as references.

DNA sequence similarity studies. The S1 nuclease hybridization technique, as described by Johnson and Palleroni (14) and Hildebrand et al. (6), was used to determine the levels of DNA sequence similarity between strain B62^T and strains belonging to other *Pseudomonas* species and pathovars of *X. campestris* (Table 1).

16S rRNA gene DNA sequencing. Genomic DNA was prepared as described above. The 16S rRNA gene sequences were amplified by using the PCR (21, 25). The PCR product was sequenced directly (1, 4). Each symmetrical PCR mixture contained buffer (50 mM KCl, 10 mM Tris [pH 8.0], 1.5 mM MgCl₂, 0.1% Triton X-100), each deoxynucleoside triphosphate at a concentration of 200 µM, 0.5 µM oligonucleotide primer 27f, 0.5 µM oligonucleotide primer 1525r (16), 2.5 U of *Taq* DNA polymerase (Promega, Madison, Wis.), and 200 ng of DNA per 100 µl of reaction mixture. The first amplification cycle consisted of denaturation at 96°C for 1.5 min, annealing at 48°C for 2 min, and elongation at 76°C for 4 min. In the 39 other cycles, the heating step was carried out at 95°C for 0.5 min. Each symmetrical PCR mixture was electrophoresed in a low-melting-point agarose gel (SeaPlaque agarose; FMC Bio-products, Rockland, Maine), and the amplification product was excised from the gel. After the preparation was melted at 65°C for 15 min, the melted gel was added (10%, vol/vol) to an asymmetrical reaction mixture which differed from the symmetrical reaction mixture by containing one of the oligonucleotide primers at a concentration of 1 µM and the other at a concentration of 0.05 µM. The asymmetrical amplification reactions were performed for 30 cycles. Both strands of DNA were asymmetrically amplified.

Both DNA strands were sequenced by using the dideoxy sequencing method (26), Sequenase enzyme (United States Biochemical, Cleveland, Ohio), oligonucleotide primers for conserved sequence regions (16), and fluorescent dideoxynucleotides. A Genesis 2000 DNA sequencer (Dupont, Wilmington, Del.) was used.

Approximately 1,450 bp of the B62^T 16S rRNA gene sequence were aligned with other 16S rRNA gene sequences obtained from GenBank, including the sequences of *Comamonas testosteroni* (accession number M11224), *Burkholderia cepacia* (accession number M22518), *P. aeruginosa* (accession number X06684), *P. mendocina* (accession number M59154), *P. fluorescens* (accession numbers D11188, D11237, and D11286), *Pseudomonas diminuta* (accession number M59064) *Escherichia coli* (accession numbers J01859, K02555, M24828, M24911, and M24996), and *Stenotrophomonas maltophilia* (22a) (accession number M59158). The sequences were aligned and similarity values were calculated by using a personal computer-based multiple-sequence screen editor written by Tommy O. Johnson (Blacksburg, Va.). The unweighted pair group method of cluster analysis (28) was used to generate a dendrogram.

Nucleotide sequence accession number. The DNA sequence obtained in this study has been deposited in the GenBank data base under accession number U01916.

RESULTS

Biochemical, nutritional, and flagellum characteristics. The two strains of the new species gave identical results in all tests.

FIG. 1. Electron micrograph of a cell of *P. flavescens* NCPPB 3063^T, showing the single polar flagellum. Magnification, $\times 12,000$.

Their properties are given below in the species description. A single polar flagellum was observed by transmission electron microscopy (Fig. 1).

Plant inoculation tests. When inoculated by themselves, the strains did not cause any damage to plants, aside from the injury caused by the inoculation process. The bacteria could be reisolated from injured areas 1 month after inoculation. When the strains were coinoculated with *X. campestris* pv. *juglandis*, the lesions were typical of those caused by *X. campestris* pv. *juglandis*. Yellow bacteria which resembled strain B62^T were also reisolated from the lesions 1 month after inoculation. Yellow ooze was not observed around any of the stem inoculation areas.

Pigment analysis. A spectral analysis of the yellow cellular pigment indicated that it was different from the xanthomonads characteristic of the genus *Xanthomonas*. Under the conditions of our analyses, the position of the major peak of pigment extracted with methanol from the xanthomonad reference strains ranged from 442 to 443.5 nm, whereas the major peak of the pigment of the new species was at 446 nm.

A spectral analysis of the supernatants of cultures grown in King medium B broth, a medium which induces production of the fluorescent pigment, revealed a single peak at 405 nm for strains B62^T and B62-D5. Single absorption peaks were observed for *P. mendocina*, *P. cichorii*, *P. syringae*, *P. putida*, *P. fluorescens*, and *P. aeruginosa* at 370, 408, 410, 418, 422, and 448 nm, respectively.

DNA base composition. The genomic DNAs of strains B62^T and B62-D5 had a base composition of 63 mol% G+C.

DNA hybridization experiments. DNA sequence similarity was not detectable when DNA from B62^T and B62-D5 was hybridized with DNA from *S. maltophilia* or with DNA from *X. campestris* pv. *campestris*, *X. campestris* pv. *pelargonii*, *X. campestris* pv. *phaseoli*, *X. campestris* pv. *oryzicola*, or *X. campestris* pv. *vesicatoria*. The levels of similarity with *X. campestris* pv. *begoniae*, *X. campestris* pv. *celebensis*, *X. campestris* pv. *glycines*, *X. campestris* pv. *juglandis*, *X. campestris* pv. *taraxaci*, and *X. campestris* pv. *vitians* ranged from 1 to 5%.

Levels of similarity between B62^T DNA and DNAs from organisms belonging to *Pseudomonas* rRNA groups I, II, and III were low (Table 2). The organisms tested included nine *Pseudomonas* rRNA group I species, *B. cepacia* (rRNA group II), and *C. testosteroni* (rRNA group III). The levels of similarity between B62^T and members of the genera *Alcaligenes*, *Acidovorax*, *Hydrogenophaga*, and *Serratia* (Table 1) were less than 1%.

16S rRNA sequencing. The 16S rRNA sequence of strain B62^T (data not shown) was compared with the sequences of other organisms (Table 3). The 16S ribosomal DNA sequence similarity values ranged from 95.18 to 97.8% with the three

TABLE 2. Levels of DNA similarity between *P. flavescens* and various strains of *Pseudomonas*, *Burkholderia*, and *Comamonas* species

Strain	% DNA sequence similarity
<i>P. aeruginosa</i> ATCC 10145 ^T	11
<i>P. alcaligenes</i> ATCC 14909 ^T	11
<i>P. mendocina</i> ATCC 25411 ^T	11
<i>P. agarici</i> ATCC 25943.....	6
<i>P. synxantha</i> ATCC 9890 ^T	7
<i>P. fluorescens</i> ATCC 13525 ^T	7
<i>P. chlororaphis</i> ATCC 9446 ^T	11
<i>P. putida</i> ATCC 12633 ^T	9
<i>P. syringae</i> ATCC 19304 ^T	7
<i>B. cepacia</i> ATCC 25416 ^T	11
<i>C. testosteroni</i> ATCC 11996 ^T	4
<i>C. testosteroni</i> ATCC 17510.....	9

rRNA group I species tested (*P. aeruginosa*, *P. mendocina*, and *P. fluorescens*). The similarity values were significantly lower with rRNA sequences from members of rRNA homology group II (*B. cepacia*), group III (*C. testosteroni*), group IV (*P. diminuta*), and group V (*S. maltophilia*). A dendrogram illustrating these results is shown in Fig. 2.

DISCUSSION

The results of DNA hybridization studies performed with strains B62^T and B62-D5 and a comparison of the B62^T 16S rRNA gene sequence with the sequences of a number of *Xanthomonas* and *Pseudomonas* species indicated that the new organism should be placed in the genus *Pseudomonas*. In addition, B62^T and B62-D5 produce a fluorescent pigment on King's medium B (15), which is characteristic of many rRNA group I pseudomonad species. However, the nutritional characteristics of strains B62^T and B62-D5 (Table 4) and the low levels of DNA similarity with *Pseudomonas* rRNA homology group I, II, and III species and with *Pseudomonas agarici* and *Pseudomonas synxantha* (Table 2) showed that strains B62^T and B62-D5 are quite distinct from other pseudomonads and thus should be designated a new species. These plant-associated strains share many properties with the major groups of phytopathogenic fluorescent pseudomonads (27). They lack the arginine dihydrolase system, but they are oxidase positive. The only other fluorescent pseudomonad known to give similar results in these tests is *P. cichorii*. Overall, the nutritional properties of the new strains are similar to those of *Pseudomonas viridiflava* and *P. syringae* pv. *syringae*; the exceptions to this are the capacity of the new strains to utilize itaconate, mesaconate, trehalose, and ethanolamine and their failure to utilize ribose, erythritol, and inositol. Another physiological similarity to the phytopathogenic strains is in the growth rates, which are slower than those of most saprophytic fluorescent pseudomonads (7). The similarities in nutritional properties to other phytopathogenic species suggest the possibility that some of these properties may be related to the capacity to grow in plant tissue, a possibility that deserves further investigation. Despite these findings, the level of DNA similarity between *P. flavescens* and *P. syringae* was only 7%. It has been shown previously by using the S1 nuclease and membrane DNA hybridization methods, respectively, that *P. syringae* exhibits 23% (14) and 49% (24) DNA similarity with *P. cichorii*. It is well known that the S1 nuclease method for analyzing levels of DNA similarity results in lower DNA homology values than the membrane methods do (13). Levels of DNA similarity of

TABLE 3. Levels of sequence similarity for aligned 16S rRNA genes

Organism	Accession no.	% Sequence similarity to:								
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>P. mendocina</i>	<i>P. fluorescens</i>	<i>P. fluorescens</i> B62 ^T	<i>B. cepacia</i>	<i>C. testosteroni</i>	<i>P. diminuta</i>	<i>S. maltophilia</i>
<i>E. coli</i>	— ^a	100	87.06	86.63	85.50	87.10	81.16	81.18	80.35	82.04
<i>P. aeruginosa</i>	X06684		100	92.26	95.78	95.18	84.14	83.17	80.23	83.63
<i>P. mendocina</i>	M59154			100	96.89	97.80	84.83	83.69	81.26	86.35
<i>P. fluorescens</i>	— ^b				100	96.28	84.72	83.01	82.31	82.89
<i>P. fluorescens</i> B62 ^T	U01916					100	84.87	84.45	81.22	84.99
<i>B. cepacia</i>	M22518						100	89.14	80.52	84.47
<i>C. testosteroni</i>	M11224							100	80.52	82.31
<i>P. diminuta</i>	M59064								100	80.55
<i>S. maltophilia</i>	M59158									100

^a Composite of GenBank files J01859, K02555, M24828, M24911, and M24996.

^b Composite of GenBank files D11188, D11237, and D11286.

37 to 41% were found for *P. syringae* and *P. viridiflava* (24) by using the membrane method. Since *P. syringae* DNA similarity values were higher with *P. cichorii* and *P. viridiflava* than with strains B62^T and B62-D5, it is unlikely that B62^T and B62-D5 are closely related to *P. cichorii* and *P. viridiflava*. Data from mathematical models (8, 9) indicated that this assumption is correct.

The yellow cellular pigment isolated from B62^T and B62-D5 was different from the pigments of the xanthomonads tested. The pigments produced by xanthomonads are unique and consist of brominated aryl-polyenes with an absorption peak at 443 nm (11). The yellow pigment also differed from the carotenoid pigment (22) produced by nonfluorescent *P. mendocina* (data not shown). Strains B62^T and B62-D5 produced a fluorescent pigment with an absorption peak at 405 nm.

A *Pseudomonas* species similar in some phenotypic properties to the organism described in this paper was proposed under the name *Pseudomonas flavida* by Itoh et al. (12). The

strain studied by Itoh et al. was isolated from an enrichment culture by using caffeine. The colonies of this organism are yellow, and the cultures also produce a fluorescent pigment. *P. flavida* differs from our strains in being able to utilize caffeine, in having a negative oxidase reaction, in accumulating poly-β-hydroxybutyrate, and in giving positive arginine dihydrolase and gelatinase reactions. The production of a fluorescent pigment suggests that *P. flavida* is a member of the genus *Pseudomonas* sensu stricto (rRNA homology group I). The levels of DNA similarity between strain IF-4 of *P. flavida* (kindly sent to us by N. Itoh) and strains B62^T and B62-D5 were determined by using DNA competition and S1 nuclease methods. In both cases the level of homology was 0%. This finding supports the conclusion that *P. flavida* is different from the species described in this paper.

The results of a comparison of the rRNA gene sequence of strain B62^T with the rRNA gene sequences of *Pseudomonas* species belonging to rRNA homology groups I to V indicated that B62^T is related to rRNA group I. The most similar sequence was that of *P. mendocina*. This suggests that B62^T should be placed in rRNA homology group I. On the basis of all of the properties which we examined, we concluded that strains B62^T and B62-D5 belong to a new species of the genus *Pseudomonas*, for which we propose the name *Pseudomonas flavescens* sp. nov.

Description of *Pseudomonas flavescens* sp. nov. *Pseudomonas flavescens* (fla.ves'cens. L.v. *flavescens*, to become golden yellow; L. part. adj. *flavescens*, becoming golden yellow). Both strains that have been isolated are aerobic, gram negative, and oxidase positive. The cells are slightly curved rods (0.6 to 0.7 by 1.6 to 2.3 μm) and are motile by means of a single polar flagellum (Fig. 1). They are negative for arginine dihydrolase activity, ice nucleation, and hypersensitivity.

They do not denitrify, nor do they accumulate poly-β-hydroxybutyrate. Hydrolysis of gelatin, starch, and Tween 80 is negative, and growth does not occur at 37°C. Colonies are yellow on YDCP medium and King medium B, and a diffusible fluorescent pigment is produced on King medium B. β-Glucosidase, rutin glycosidase, and pectinolytic enzymes are not produced. These organisms do not cause soft rot of potatoes and do not produce levan. Growth factors are not required. The following compounds are utilized for growth: acetate, *cis*-aconitate, D-alanine, L-alanine, α-aminobutyrate, L-arabi-

FIG. 2. Dendrogram showing the relationships among *P. flavescens*, *E. coli*, and strains belonging to *Pseudomonas* rRNA group I (*P. mendocina*, *P. fluorescens*, and *P. aeruginosa*), rRNA group II (*B. cepacia*), rRNA group III (*C. testosteroni*), rRNA group IV (*P. diminuta*), and rRNA group V (*S. maltophilia*). The dendrogram is based on levels of similarity of the 16S rRNA gene sequences. The unweighted pair method of Sokal and Sneath (28) was used to generate the dendrogram.

TABLE 4. Characteristics of *P. flavescentis* and other *Pseudomonas* species

Characteristic	<i>P. flavescentis</i>	<i>P. syringae</i> ^a	<i>P. viridiflava</i> ^a	<i>P. cichorii</i> ^a	<i>P. fluorescens</i> ^b	<i>P. aureofaciens</i> ^b	<i>P. chlororaphis</i> ^b	<i>P. putida</i> ^b	<i>P. aeruginosa</i>	<i>P. mendocina</i>
No. of flagella	1	>1	1-2	>1	>1	>1	>1	>1	1	1
Growth at 42°C	— ^c	—	—	—	—	—	—	—	—	+
Yellow cellular pigment	+	—	—	—	—	—	—	—	—	+
Oxidase	+	—	—	+	+	+	+	+	+	+
Arginine dihydrolase	—	—	—	—	+	+	+	+	+	+
Gelatin liquefaction	—	d	+	—	+	+	+	—	+	—
Hypersensitive response	—	+	+	+	—	—	—	—	—	—
Denitrification	—	—	—	—	d	—	+	—	+	+
Utilization of:										
β-Alanine	—	—	—	—	+	+	+	+	+	+
D-Aspartate	—	—	—	+	d	—	d	d	—	—
Benzoate	—	—	—	—	d	d	+	d	+	d
Erythritol	—	d	+	—	d	—	—	—	—	—
Ethanol	—	—	—	—	d	—	—	d	+	+
Geraniol	—	—	—	—	—	—	—	—	+	+
Hydroxymethylglutarate	+	d	+	+	d	—	—	—	—	d
Isoleucine	—	—	—	—	+	+	+	+	d	+
L-Lysine	—	—	—	—	d	d	d	d	+	—
D-Malate	+	d	+	+	d	—	d	d	d	d
Ribose	—	d	+	+	d	+	+	d	+	—
Isovalerate	—	—	—	—	d	+	+	+	+	+
D-(−)-Tartrate	—	d	+	—	d	—	—	d	—	—
L-(+)-Tartrate	—	d	—	+	d	—	d	d	—	d
<i>m</i> -Tartrate	+	d	+	+	d	—	—	d	—	—
Trehalose	+	—	—	—	d	d	+	—	—	—
Trigonelline	+	d	+	+	d	—	—	d	—	—
L-Tyrosine	—	d	+	+	+	+	+	+	+	+
L-Valine	—	—	—	—	+	+	+	+	d	+

^a Data from this study and the studies of Hildebrand and Schroth (7), Hildebrand et al. (9), and Palleroni (22).^b Data from this study and the studies of Stanier et al. (29) and Palleroni (22).^c d, 11 to 89% of the strains are positive; +, 90% or more of the strains are positive; —, 90% or more of the strains are negative.

nose, L-asparagine, L-aspartate, betaine, 2,3-butanediol, caprate, caproate (slowly), caprylate, choline, citrate, citrulline, creatine, ethanolamine, fumarate, galactose, galacturonate, gluconate, glucose, glutamate, glycerate (slowly), glycerol, glycolate, heptanoate, L-histidine, *p*-hydroxybenzoate, β-hydroxybutyrate, β-hydroxymethylglutarate, itaconate, α-ketoglutarate, L-lactate, L-leucine, D-malate, L-malate, malonate, mannitol, mannose, mesaconate, mucate, pelargonate, proline, pyruvate, quinate, saccharate, serine (slowly), succinate, sucrose, *m*-tartrate, trehalose, trigonelline, and xylose. The substances not utilized for growth include acetamide, *trans*-aconitate, adipate, adonitol, β-alanine, α-aminobutyrate, α-aminovalerate, δ-aminovalerate, α-amylamine, anthranilate, D-arabinose, L-arabitol, L-arginine, D-aspartate, azelate, benzoate, benzoylformate, benzylamine, butanol, butylamine, butyrate, cellobiose, citraconate, *n*-dodecane, dulcitol, erythritol, ethanol, ethylene glycol, D-fucose, geraniol, glycine, *n*-hexadecane, hippurate, histamine, homoserine, *m*-hydroxybenzoate, *o*-hydroxybenzoate, inositol, insulin, isobutanol, isobutyrate, isoleucine, isovalerate, 2-ketogluconate, kynurenate, kynurenine, lactose, levulinate, L-lysine, maltose, D-mandelate, L-mandelate, melibiose, naphthalene, nicotinate, norleucine, pantothenate, phenol, phenylacetate, phenylalanine, phthalate, pimelate, *n*-propanol, propionate, propylene glycol, ornithine, L-rhamnose, ribose, rutin, salicin, sebacate, sorbitol, sorbose, spermine, starch, suberate, D-(−)-tartrate, L-(+)-tartrate, testosterone, L-threonine, D-tryptophan, L-tryptophan, L-tyrosine, and L-valine.

The base composition of the DNA is 63 mol% G+C. The type strain is NCPPB 3063.

ACKNOWLEDGMENT

We are indebted to Robert L. Gherna of the American Type Culture Collection for his generous help in supplying bacterial strains for comparative studies.

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