Taxonomy of *Pseudomonas* Strains Isolated from Tomato Pith Necrosis: Emended Description of *Pseudomonas corrugata* and Proposal of Three Unnamed Fluorescent *Pseudomonas* Genomospecies

L. SUTRA,^{1,2} F. SIVERIO,³ M. M. LOPEZ,⁴ G. HUNAUULT,² C. BOLLET,⁵ AND L. GARDAN^{1*}

Institut National de la Recherche Agronomique, Station de Pathologie Végétale et Phytobactériologie, Beaucouzé,¹
Université d'Angers, Faculté des Sciences, Angers,² and Antimicrobiens et Facteurs de Colonisation dans les
Pathologies Transmissibles, Faculté de Pharmacie, Marseille,⁵ France; and Instituto Canario de
Investigaciones Agrarias, La Laguna, Tenerife,³ and Instituto Valenciano
de Investigaciones Agrarias, Valencia,⁴ Spain

Thirty-three fluorescent *Pseudomonas* strains isolated from tomato pith necrosis (FPTPN strains) and 89 *Pseudomonas corrugata* strains were studied by numerical taxonomy. In the dendrogram of distances, the *P. corrugata* strains constituted a single phenon (phenon 1), whereas 17 of the 33 FPTPN strains clustered in a separate phenon (phenon 2). The other 16 FPTPN strains were included in phenon 1 consisting of well-characterized fluorescent *Pseudomonas* species or were isolated phenotypes. Phenon 1 and 2 were distinguished by fluorescence on King B medium, accumulation of poly- β -hydroxybutyrate, production of levan, and assimilation of sorbitol. DNA-DNA hybridization showed that *P. corrugata* is a true genomic species (66 to 100% DNA relatedness) and that the FPTPN strains of phenon 2 were divided into three genomic groups. Genomic groups 1 and 2 were not distinct from each other phenotypically, and genomic group 3 could be distinguished from genomic groups 1 and 2 only on the basis of assimilation of citraconate and laevulinate. Genomic groups 1 and 2 are related to *P. corrugata* (40 to 55% DNA relatedness), whereas genomic group 3 is less closely related to *P. corrugata* (20 to 23% DNA relatedness). The lipopolysaccharide patterns on electrophoresis gels and fatty acid profiles of strains belonging to genomic groups 1 through 3 are different from each other and from the lipopolysaccharide patterns and fatty acid profiles of *P. corrugata*. However, cross-reactions were observed between *P. corrugata* and the FPTPN strain genomic groups, indicating that there are common epitopes of the lipopolysaccharides. The three FPTPN strain genomic groups were not named as species but were designated *Pseudomonas* genomospecies FP1, FP2, and FP3.

Pseudomonas corrugata Roberts and Scarlett 1981 was first described by Scarlett et al. (35) as the causal agent of tomato pith necrosis. *P. corrugata* has been isolated all over the world and causes pith necrosis mainly in tomato (2, 7, 8, 15, 21, 23, 25, 32, 35), but also in pepper (25) and chrysanthemum (14). This bacterium has also been identified in symptomless alfalfa plants (26), soil (37), and rice grains (45). Several authors have indicated that it is a ubiquitous bacterium with a wide range of host plants, a common characteristic of many opportunistic fluorescent pseudomonads (26, 35, 38).

In the original description based on a limited number of phenotypic and pathogenic characteristics (35), *P. corrugata* was considered a nonfluorescent *Pseudomonas* species that accumulates poly- β -hydroxybutyrate (PHB). Consequently, Palleroni (31) included *P. corrugata* in section V of the genus *Pseudomonas* among the nonfluorescent bacteria now classified in the genera *Pseudomonas*, *Burkholderia*, and *Acidovorax*. On the basis of DNA-rRNA hybridization data, De Vos et al. (12) included *P. corrugata* in the *Pseudomonas fluorescens* rRNA group, among the fluorescent pseudomonads. A similar conclusion was reached by Stead (42), who grouped plant-pathogenic pseudomonads and other species of pseudomonads on the basis of their fatty acid profiles and placed *P. corrugata*

in group 1, which contained six subgroups. One of these subgroups, subgroup 1a, corresponded to the fluorescent pseudomonads, and subgroup 1b corresponded to *P. corrugata* (it was the only member of this subgroup). Hu et al. (19), who performed a numerical analysis of biochemical and nutritional properties, recognized *P. corrugata* as a distinct species of the genus *Pseudomonas*. Young et al. (48) included *P. corrugata* in the fluorescent group, but considered it to be anomalous, because it is nonfluorescent and PHB positive. Recent studies performed with a collection of *P. corrugata* strains from different hosts and countries demonstrated that there is a great deal of heterogeneity in the biochemical and serological characteristics of these organisms (38, 39) and in their fatty acid profiles (40).

Several fluorescent pseudomonads have also been identified in different countries as causal agents of tomato pith necrosis; these organisms include *Pseudomonas cichorii* in New Zealand (47) and unidentified fluorescent pseudomonads in Greece, Canada, and Italy (3, 6, 13). The relatively frequent isolation of fluorescent *Pseudomonas* strains from tomato pith necrosis (FPTPN strains) in France and Spain prompted us to study the characteristics of these isolates in comparison with the characteristics of *P. corrugata*.

The aims of this work were (i) to improve our knowledge of the phenotypic diversity of *P. corrugata* strains, (ii) to compare *P. corrugata* strains with FPTPN strains, and (iii) to study the genomic homology among strains of *P. corrugata* and FPTPN strains and between the two groups. To do this, we used a polyphasic approach, which included a numerical taxonomy

* Corresponding author. Mailing address: Institut National de la Recherche Agronomique, Station de Pathologie Végétale et Phytobactériologie, BP 47, 42 rue Georges Morel, 49071 Beaucouzé Cedex, France. Phone: (33) 02 41 22 57 29. Fax: (33) 02 41 22 57 05. E-mail: gardan@angers.inra.fr.

TABLE 1. Distribution of strains among the phena and isolated phenotypes

Phenon or group	Subphenon or subgroup	No. of strains	Strain(s)
1	1a	63	<i>Pseudomonas corrugata</i> CFBP 2431 ^T ; <i>Pseudomonas corrugata</i> strains isolated from tomato (59 strains), pepper (1 strain), and lucerne (2 strains)
	1b	20	<i>Pseudomonas corrugata</i> strains isolated from tomato (18 strains) and pepper (1 strain); FPTPN strain CFBP 10552
		6	Isolated phenotypes: <i>Pseudomonas corrugata</i> CFBP 10143, CFBP 10560, CFBP 10902, CFBP 10903, CFBP 10930, and CFBP 10937
2	2a	8	FPTPN strains CFBP 10435, CFBP 10437, CFBP 10529, CFBP 10604, CFBP 10605, CFBP 10934, and CFBP 11062; <i>Pseudomonas corrugata</i> CFBP 10436
	2b	6	FPTPN strains CFBP 10530, CFBP 10531, CFBP 10533, CFBP 10551, CFBP 10567, and CFBP 10591
	2c	3	FPTPN strains CFBP 10524, CFBP 10569, and CFBP 10583
		1	Isolated phenotype: FPTPN strain CFBP 10535
3		2	<i>Pseudomonas fluorescens</i> biotype II strain CFBP 2125; <i>Pseudomonas fluorescens</i> biotype IV strain CFBP 2129
4		10	<i>Pseudomonas fuscovaginae</i> CFBP 2065 ^T , CFBP 2801, CFBP 2802, CFBP 2803, CFBP 2805, CFBP 2808, CFBP 3081, CFBP 3082, CFBP 3083, and CFBP 3084
5		14	<i>Pseudomonas marginalis</i> pv. <i>marginalis</i> CFBP 10051, CFBP 10053, CFBP 10054, CFBP 10061, CFBP 10068, and CFBP 1538; <i>Pseudomonas marginalis</i> pv. <i>pastinacae</i> CFBP 2038 ^T ; <i>Pseudomonas marginalis</i> pv. <i>alfalfae</i> CFBP 2039 ^T ; <i>Pseudomonas fluorescens</i> biovar I strain CFBP 2123; FPTPN strains CFBP 10537, CFBP 10539, CFBP 10549, CFBP 10562, and CFBP 10563
6		11	<i>Pseudomonas</i> sp. strain CFBP 2022; <i>Pseudomonas fluorescens</i> biovar I strain CFBP 2102 ^T ; <i>Pseudomonas tolaasii</i> CFBP 2068 ^T , CFBP 2810, CFBP 10046, CFBP 10047, CFBP 10071, CFBP 10074, CFBP 10075, and CFBP 10076; FPTPN strain CFBP 10582
7		3	<i>Pseudomonas asplenii</i> CFBP 3279; " <i>Pseudomonas blatchfordae</i> " CFBP 3280; <i>Pseudomonas flectens</i> CFBP 3281 ^T
8		3	<i>Pseudomonas aeruginosa</i> CFBP 2466 ^T ; FPTPN strains CFBP 10601 and CFBP 10602
9		2	<i>Pseudomonas chlororaphis</i> CFBP 2132 ^T ; <i>Pseudomonas aureofaciens</i> CFBP 2133 ^T
10		2	<i>Pseudomonas putida</i> CFBP 2066 ^T and CFBP 3142.1
11		11	<i>Pseudomonas cichorii</i> CFBP 2101 ^T , CFBP 1149, CFBP 1474, CFBP 1477, CFBP 11417, CFBP 11420, CFBP 11424, CFBP 11431, CFBP 11434, CFBP 11435, and CFBP 11443
12		2	FPTPN strains CFBP 10960 and CFBP 10962
13		2	<i>Burkholderia gladioli</i> pv. <i>allicola</i> CFBP 2422 ^T and <i>Burkholderia gladioli</i> pv. <i>gladioli</i> CFBP 2427 ^T
14		2	<i>Acidovorax avenae</i> subsp. <i>avenae</i> CFBP 2425 ^T and CFBP 2426
Isolated phenotypes		25	FPTPN strains CFBP 10536, CFBP 10538, and CFBP 10595; <i>Pseudomonas marginalis</i> CFBP 3300 ^T ; <i>Pseudomonas fluorescens</i> biotype II strain CFBP 2127; <i>Pseudomonas fluorescens</i> biotype V strain CFBP 2130; FPTPN strain CFBP 10534; <i>Pseudomonas putida</i> CFBP 3140.1; <i>Pseudomonas cichorii</i> CFBP 11442; <i>Burkholderia cepacia</i> CFBP 2227 ^T ; <i>Burkholderia glumae</i> CFBP 2430 ^T ; <i>Burkholderia plantarii</i> CFBP 3199 ^T ; <i>Herbaspirillum rubrisubalbicans</i> CFBP 1202; <i>Pseudomonas caricapapayae</i> CFBP 3204; <i>Burkholderia caryophylli</i> CFBP 2429 ^T ; <i>Acidovorax avenae</i> subsp. <i>avenae</i> CFBP 1201 ^T ; <i>Acidovorax avenae</i> subsp. <i>cattleyae</i> CFBP 2423 ^T ; <i>Pseudomonas agarici</i> CFBP 2063 ^T ; <i>Pseudomonas stutzeri</i> CFBP 2443 ^T ; <i>Ralstonia solanacearum</i> CFBP 2047 ^T ; <i>Pseudomonas pseudoalcaligenes</i> CFBP 2435 ^T ; <i>Burkholderia andropogonis</i> CFBP 2421 ^T ; <i>Pseudomonas meliae</i> CFBP 3225 ^T ; FPTPN strain CFBP 10927; <i>Pseudomonas cissicola</i> CFBP 2432 ^T

analysis based on data from phenotypic tests, DNA-DNA hybridizations, fatty acid profiles, lipopolysaccharide (LPS) patterns in electrophoresis gels, serology, and pathogenicity tests.

MATERIALS AND METHODS

Bacterial strains. A total of 196 strains were studied (Table 1). The first set of strains included 33 FPTPN strains isolated from 1980 to 1990 in France (24 strains), Spain (5 strains), Switzerland (2 strains), and Canada (2 strains). The two strains from Canada (CFBP 10435 and CFBP 10437) were previously described as novel agents of tomato stem necrosis (13). The strains in the second set of strains (89 *P. corrugata* strains) were isolated from tomato (85 strains), pepper (2 strains), and lucerne (2 strains) in the following 11 countries: Spain (31 strains), France (17 strains), New Zealand (9 strains), United Kingdom (8 strains), United States (8 strains), Italy (4 strains), Switzerland (4 strains), Japan (3 strains), Germany (2 strains), Sweden (2 strains), and Canada (1 strain). The

third set of strains included 74 type strains or well-characterized strains of fluorescent and nonfluorescent *Pseudomonas*, *Acidovorax*, *Burkholderia*, *Herbaspirillum*, and *Ralstonia* species, used as reference strains for numerical taxonomy and DNA-DNA hybridizations.

All of the bacteria were cultured routinely on YBGA (yeast extract, 7 g; Bacto Peptone [Difco], 7 g; glucose, 7 g; agar, 15 g; distilled water, 1,000 ml; pH 7.2) and incubated at 25°C.

Biochemical and physiological tests. The presence of oxidase, gelatinase, and arginine dihydrolase activities, production of fluorescent pigment on King B medium, levan formation, acid production from sucrose, sorbitol, erythritol, and mannitol, reduction of nitrates, hydrolysis of Tween 80 and esculin, and the hypersensitivity reaction on tobacco leaves were tested as described by Leliott et al. (24). Pectolytic activity was tested by using the method of Prunier and Kaiser (33) and Hildebrand (17). Utilization of lactate, L-(+)-tartrate, and D-(-)-tartrate was tested on the basal medium of Ayers et al. (4) supplemented with 0.1% (wt/vol) organic acid sodium salts. The presence of DNase was tested on

DNA agar (Diagnostic Pasteur, Marnes la Coquette, France). Assimilation of 49 carbohydrates, 49 organic acids, and 49 amino acids was studied by using API 50CH, API 50AO, and API 50AA strips (BioMérieux, La Balme les Grottes, France), respectively. The accumulation of granules of PHB was tested by the method of Ostle and Holt (29) by using Nile blue A; *Ralstonia solanacearum* CFBP 2047^T was used as a positive control. Flagella were stained by the method of Rhodes (34).

Numerical taxonomy. A total of 167 characteristics were included in the numerical taxonomy analysis. A distance matrix was calculated by using the Jaccard coefficient (41). Cluster analysis was done by using the unweighted pair group method with averages. At a given distance level and for the different phenotypes, the amount of information for each test was measured by calculating the diagnostic ability coefficient to determine the discriminating biochemical characteristics of the strains (10).

DNA extraction and purification. Extraction and purification of DNA were performed by using previously described methods (5).

DNA-DNA hybridization. Native DNAs were labeled in vitro by nick translation with tritium-labeled nucleotides (Amersham International, Amersham, England). The S1 nuclease-trichloroacetic acid method used for hybridization has been described previously (9, 16). The reassociation temperature was 70°C. DNA-DNA hybridization tests were carried out by using labeled DNAs from *P. corrugata* CFBP 2431^T and from three FPTPN strains (CFBP 10529, CFBP 10530, and CFBP 10569). The strains used in DNA-DNA hybridizations are listed in Tables 4 and 5. DNA-DNA hybridization tests were repeated at least twice.

Thermal stability of reassociated DNA. The temperature at which 50% of reassociated DNA became hydrolyzable by nuclease S1 (T_m) was determined by using the method of Crosa et al. (9). The T_m was determined only if the level of DNA-DNA hybridization was between 65 and 70%.

DNA base composition. The guanine-plus-cytosine (G+C) contents of *P. corrugata* CFBP 2431^T and FPTPN strains CFBP 10529, CFBP 10530, and CFBP 10569 were determined by the thermal denaturation temperature method (27) and were calculated by using the equation of Owen and Lapege (30).

Serological techniques. Six rabbit antisera were prepared with bacterial somatic antigens and were used as described by Siverio et al. (39). Three antisera were raised against *P. corrugata* CFBP 2431^T, CFBP 10147, and CFBP 10148, and the characteristics of these antisera have been studied previously (39). Three other antisera were prepared against FPTPN strains CFBP 10529, CFBP 10530, and CFBP 10569.

An indirect enzyme-linked immunosorbent assay (ELISA-I) was used to determine serological relationships among *P. corrugata* and FPTPN strains. The technique used has been described by Siverio et al. (39). Two replicates consisting of four wells in two different plates were coated with each bacterial isolate. Standard positive and negative controls were included in each plate to make comparisons between plates feasible. The positive control was a mixture of suspensions (10^7 CFU/ml in carbonate buffer, pH 9.6) of the six strains homologous to the six antisera used. The control wells were subsequently treated with a mixture of the six antisera at working dilutions (1:25,000 for the serum against strain CFBP 2431^T; 1:8,000 for the serum against strain CFBP 10147; 1:5,000 for the serum against strain CFBP 10148; and 1:10,000 for the sera against strains CFBP 10529, CFBP 10530, and CFBP 10569). A reaction was considered positive when the absorbance was twice that of the negative control in each plate.

LPS patterns. Preparation of whole-cell bacterial lysates, proteinase K hydrolysis, polyacrylamide gel electrophoresis, and silver staining were performed with 15 *P. corrugata* strains and 18 FPTPN strains by the methods of Alarcon et al. (1), Hitchcock and Brown (18), Laemmli (22), and Tsai and Frasch (44), respectively, as described in detail elsewhere (39). LPS profiles were determined at least twice for each strain analyzed.

FAME profiles. The fatty acid methyl ester (FAME) profiles of 16 *P. corrugata* strains and 17 FPTPN strains were determined by the standardized technique described by Miller and Berger (28) by using the Microbial Identification System (Hewlett-Packard model 5898 instrument) controlled with the Microbial Identification System software (MIDI; Microbial ID, Inc., Newark, Del.). The unweighted pair group method with averages was used to perform a cluster analysis of the FAME profiles.

Pathogenicity tests. All of the plants used were grown in a greenhouse. In the first experiment, the pathogenicity of a some of 30 *P. corrugata* strains and 17 FPTPN strains for tomato (*Lycopersicon esculentum* cv. INRA Montfavet 63.4) was tested by using 2-month-old plants (five plants per bacterial strain). Bacterial suspensions (about 10^7 CFU/ml) in sterile water were injected into the stems with a hypodermic needle at two points (about 30 cm and 1 m above the soil). Symptoms were recorded for up to 8 weeks after inoculation; each plant was longitudinally cut, and the length of the pith necrosis was measured.

In the second experiment, 1-month-old plants of tomato, tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*), bean (*Phaseolus vulgaris*), cauliflower (*Brassica oleracea* var. botrytis), melon (*Cucumis sativus*), and zucchini (*Cucurbita pepo*) were used. One inoculation (about 10 cm above the soil) was performed as described above with *P. corrugata* CFBP 2431^T and CFBP 10148 and with FPTPN strains CFBP 10529, CFBP 10530, CFBP 10569, and CFBP 10934. After inoculation, the plants were kept for 3 days at a relative humidity of 100% and then at 20 to 25°C at a relative humidity of 70%. Observations were made for up to 4 weeks after inoculation; every plant was longitudinally cut, and the length of

the pith necrosis was measured. For each plant species tested, reisolation of bacteria was done on YBGA or on King B agar. In each experiment, 5 to 10 control plants were inoculated with sterile distilled water.

RESULTS

Numerical analysis of phenotypic characteristics. A dendrogram displaying the distance relationships among the 196 strains included in this study is shown in Fig. 1. At a distance of 0.2025, 14 phenotypes and 25 isolated phenotypes were observed. The distribution of strains in the different phenotypes (clusters with at least two strains) and isolated phenotypes is shown in Table 1. Six major phenotypes containing more than 10 strains (phenotype 1, 2, 4 through 6, and 11) were observed.

Phenotype 1 contained all of the 89 *P. corrugata* strains except strain CFBP 10436 and included type strain CFBP 2431 and FPTPN strain CFBP 10552.

Phenotype 2 included 17 FPTPN strains and one *P. corrugata* strain, CFBP 10436.

Phenotype 4 contained 10 *Pseudomonas fuscovaginae* strains, including type strain CFBP 2065.

Phenotype 5 contained 14 fluorescent oxidase-positive *Pseudomonas* strains: 8 strains of *Pseudomonas marginalis* pathovars, including type strain CFBP 3300, *P. fluorescens* biotype I strain CFBP 2123, and FPTPN strains CFBP 10537, CFBP 10539, CFBP 10549, CFBP 10562, and CFBP 10563.

Phenotype 6 contained the following 11 fluorescent oxidase-positive *Pseudomonas* strains: eight *Pseudomonas tolaasii* strains, including type strain CFBP 2068, *P. fluorescens* biotype I strain CFBP 2102^T, *P. fluorescens* CFBP 2022, and FPTPN strain CFBP 10582.

Phenotype 11 contained 11 *P. cichorii* strains including type strain CFBP 2101.

Eight minor phenotypes (phenotype 3, 7 through 10, and 12 through 14) each contained two or three strains. Phenotype 3 contained two strains of *P. fluorescens* biotypes II and IV, phenotype 8 contained *Pseudomonas aeruginosa* CFBP 2466^T and FPTPN strains CFBP 10601 and CFBP 10602, phenotype 10 contained *Pseudomonas putida* CFBP 2066^T and CFBP 3142.1, and phenotype 12 contained FPTPN strains CFBP 10960 and CFBP 10962. The other minor phenotypes (phenotype 7, 9, 13, and 14) included the type strains of phytopathogenic *Pseudomonas*, *Burkholderia*, or *Acidovorax* species (Table 1).

Of the 25 unclustered phenotypes at a distance of 0.2025, 12 corresponded to the type strains of fluorescent or nonfluorescent *Pseudomonas*, *Acidovorax*, *Burkholderia*, *Herbaspirillum*, and *Ralstonia* species.

The 33 FPTPN strains were distributed as follows: 1 strain in phenotype 1, 17 strains in phenotype 2, 5 strains in phenotype 5, 1 strain in phenotype 6, 2 strains in phenotype 8, and 2 strains in phenotype 12, which is closely related to *P. cichorii* (phenotype 11). Strains CFBP 10534, CFBP 10536, CFBP 10538, CFBP 10595, and CFBP 10937 appeared as isolated phenotypes (Table 1).

Biochemical and physiological characteristics that differentiate the six major phenotypes, phenotype 1, 2, 4 through 6, and 11, are shown in Table 2. The strains that clustered in phenotype 1 and 2, which were isolated from plants with tomato pith necrosis and are *P. corrugata* and FPTPN strains, respectively, are closely related phenotypically and could be distinguished only by the following four biochemical tests: production of fluorescent pigment on King B medium, accumulation of PHB, levan formation, and assimilation of sorbitol.

At a distance of 0.1755, *P. corrugata* phenotype 1 was divided into two subphenotypes, subphenotype 1a and 1b, which included 83 (93.9%) of the 89 strains, and six isolated phenotypes. The strains of subphenotype 1a and 1b can be distinguished by the

FIG. 1. Dendrogram of phenotypic distances for 196 strains. The number of strains in each phenon is indicated in Table 1.

TABLE 2. Phenotypic characteristics that differentiate phenon 1, 2, 4 through 6, and 11

Characteristic	Phenon 1 (<i>P. corrugata</i>) (n = 89) ^a	Phenon 2 (FPTPN strains) (n = 18)	Phenon 4 (<i>P. fuscovaginae</i>) (n = 10)	Phenon 5 (<i>P. marginalis</i>) (n = 14)	Phenon 6 (<i>P. tolasi</i>) (n = 11)	Phenon 11 (<i>P. cichorii</i>) (n = 11)
Fluorescence on King B medium ^b	— ^c	+	+	+	+	d(63) ^b
Levan production ^b	—	+	—	d(57) ^b	d(18) ^b	—
Arginine dihydrolase	d(77)	+	+	+	+	—
Gelatin hydrolysis	d(60)	d(55)	d(50)	d(71)	+	—
Nitrate reduction ^d	+	+	—	+	—	—
Esculin hydrolysis	—	—	—	d(27)	—	+
Hypersensitivity reaction	d(19)	—	—	—	—	+
Utilization of carbohydrates and polyalcohols						
Sucrose	+	+	—	d(78)	d(28)	—
Trehalose	+	+	+	+	+	—
L-Arabinose	+	+	+	+	d(36)	+
D-Xylose	+	+	+	+	d(34)	+
N-Acetylglucosamine	+	d(66)	—	+	+	—
Glucosamine ^d	+	+	+	+	+	—
Sorbitol ^b	—	+	—	+	+	d(18) ^b
Adonitol	—	—	—	d(71)	+	—
Erythritol	—	—	—	d(78)	+	—
Xylitol	—	—	—	d(78)	+	—
Inositol ^d	+	+	—	+	+	+
L-Arabitol	—	—	—	d(71)	+	—
Utilization of organic salts						
L-(+)-Tartrate	—	—	—	d(28)	—	+
2-Aminobenzoate ^d	—	—	+	+	+	—
meso-Tartrate	d(22)	—	+	d(78)	+	+
n-Valerate	+	+	d(50)	+	+	—
Isovalerate	d(79)	d(83)	—	+	d(27)	—
Isobutyrate	d(66)	+	d(80)	+	+	—
2-Ketogluconate	d(33)	d(72)	—	+	+	d(36)
Mesaconate ^d	—	—	—	+	+	—
Itaconate	—	d(38)	—	+	+	—
Citraconate	d(15)	d(16)	+	+	d(81)	—
Laevulinate	—	d(27)	—	d(85)	d(23)	—
Utilization of amino acids and nitrogenous compounds						
L-Tryptophan ^d	—	—	—	+	+	—
L-Valine ^d	+	+	+	+	+	—
L-Leucine ^d	+	+	+	+	+	—
L-Phenylalanine ^d	+	+	+	+	+	—
L-Citrulline	—	—	+	d(78)	d(45)	—
L-Alanine ^d	+	+	+	+	+	—
L-Isoleucine ^d	+	+	+	+	+	—
Histamine	d(26)	—	—	—	d(18)	—
Spermine ^d	+	+	—	+	+	—
Trigonelline	+	d(77)	d(80)	+	—	+
DL-Kynurenine	—	—	—	+	d(81)	—
Ethanolamine	d(77)	+	+	+	d(72)	—
Amylamine	d(13)	d(44)	—	d(57)	—	—
DL-5-Aminovalerate	+	+	—	+	d(81)	d(45)

^a n is the number of strains in the phenon.^b Characteristic that differentiates phenon 1 and 2.^c —, negative; +, 90 to 100% of the strains are positive; d, 11 to 89% of the strains are positive. The numbers in parentheses are the percentages of positive strains.^d Characteristic that clearly differentiates the six phenon.

following characteristics: assimilation of meso-tartrate, isobutyrate, and histamine (Table 3).

At a distance of 0.150, phenon 2 (FPTPN strains) was divided into three subphenon, subphenon 2a, 2b, and 2c, and one isolated phenotype (strain CFBP 10535). Subphenon 2a and 2b could not be clearly differentiated on the basis of the 167 biochemical tests used. In contrast, strains in subphenon 2c

assimilated citraconate and laevulinate, whereas strains in subphenon 2a and 2b did not.

DNA relatedness. The results of DNA-DNA hybridizations are shown in Table 4. A total of 14 *P. corrugata* strains in phenon 1 (10 strains in subphenon 1a and 4 strains in subphenon 1b) were selected for the DNA-DNA hybridization experiments. These strains demonstrated 66 to 100% DNA related-

TABLE 3. Phenotypic characteristics that differentiate phenon 1 (*P. corrugata*) and phenon 2 (FPTPN strains)

Taxon	No. of strains	Fluorescence	Levan production	Utilization of:						
				Sorbitol	meso-Tartrate	Isobutyrate	2-Ketogluconate	Citraconate	DL-Glycerate	Histamine
Phenon 1	89	— ^a	—	—	d (22)	d (66)	d (33)	d (15)	d (41)	d (26)
Subphenon 1a	63	—	—	—	—	+	d (14)	—	d (25)	—
Subphenon 1b	20	—	d (20)	—	+	—	+	d (60)	+	+
Isolated phenotypes	6									
CFBP 10143		—	—	—	—	—	—	—	+	—
CFBP 10560		—	—	—	—	—	+	—	—	—
CFBP 10902		—	—	—	—	—	—	—	+	—
CFBP 10903		—	—	—	—	+	—	—	—	—
CFBP 10930		—	+	—	—	—	—	—	+	—
CFBP 10937		—	—	—	—	+	—	—	—	—
Phenon 2	18	+	+	+	—	+	d (70)	d (17)	+	—

^a —, negative; +, positive; d, variable. The values in parentheses are percentages of positive strains.

ness to *P. corrugata* CFBP 2431^T (average, 86.3%; standard deviation, 14.9%). For the strain pairs that demonstrated the lowest DNA relatedness values (66 to 69%), the ΔT_m values ranged from 2 to 3.5°C (ΔT_m is the difference between the T_m of a homoduplex and the T_m of a heteroduplex). Strain CFBP 10436, which was a member of subphenon 2a, was 92% related to *P. corrugata* CFBP 2431^T. Thus, all 15 strains examined are considered members of a single DNA hybridization group.

The 18 FPTPN strains included in phenon 2 (17 strains) and in phenon 1 (strain CFBP 10552) were 20 to 55% related to the *P. corrugata* type strain. These strains constituted three different DNA hybridization groups (Table 4). DNA hybridization group 1 contained nine FPTPN strains which were 67 to 100% related to strain CFBP 10529 (average level of relatedness, 86.8%; standard deviation, 13.0%); DNA hybridization group 2 contained six strains which were 89 to 100% related to strain CFBP 10530 (average level of relatedness, 95.3%; standard deviation, 4.5%); and DNA hybridization group 3 contained three strains which were 86 to 100% related to strain CFBP 10569 (average level of relatedness, 93.0%; standard deviation, 7.0%) (Table 4). Most DNA hybridization group 1 and 2 strains clustered in subphenon 2a and 2b; the one exception was strain CFBP 10552, which was in subphenon 1a. The DNA hybridization group 3 strains were included in subphenon 2c.

The levels of hybridization between labeled DNA from *P. corrugata* CFBP 2431^T and DNAs from FPTPN strains belonging to DNA hybridization groups 1 and 2 on the one hand and between labeled DNAs from strains CFBP 10529 (DNA hybridization group 1) and CFBP 10530 (DNA hybridization group 2) and DNAs from *P. corrugata* strains on the other hand varied from 40 to 55%. In contrast, the levels of hybridization between labeled DNA from *P. corrugata* CFBP 2431^T and DNAs from strains belonging to DNA hybridization group 3 ranged from 20 to 23% (Table 4).

The levels of relatedness between the DNAs of *P. corrugata* CFBP 2431^T and FPTPN strains CFBP 10529, CFBP 10530, and CFBP 10569 and the DNAs of most type strains of fluorescent phytopathogenic or saprophytic oxidase-positive *Pseudomonas* species ranged from 15 to 28, 12 to 33, 14 to 36, and 10 to 48%, respectively; the one exception was the levels of reassociation with *Pseudomonas flectens* CFBP 3281^T, which were less than 2% (Table 5). The levels of relatedness between the DNAs of *P. corrugata* CFBP 2431^T and FPTPN strains CFBP 10529, CFBP 10530, and CFBP 10569 and the DNAs of the type strains of fluorescent oxidase-negative *Pseudomonas* species varied from 8 to 19%. Most levels of DNA relatedness with reference strains of nonfluorescent *Pseudomonas*, *Ac-*

idovorax, *Burkholderia*, *Herbaspirillum*, and *Ralstonia* species were less than 5%; the one exception was the levels of DNA relatedness with *Pseudomonas alcaligenes*, which ranged from 2 to 12% (Table 5).

DNA base composition. *P. corrugata* CFBP 2431^T had a DNA G+C content of 59.6 mol%. The G+C contents of FPTPN strains CFBP 10529, CFBP 10530, and CFBP 10569 were 59.2, 59.2, and 60.2 mol%, respectively.

Serological study. The results of the ELISA-I, in which three antisera prepared against *P. corrugata* strains and three antisera prepared against FPTPN strains were used, are shown in Table 6. The mean absorbance values of negative controls ranged from 0.158 to 0.230 according to the assays, whereas the mean absorbance values of positive controls varied from 1.950 to 2.246.

Antisera against *P. corrugata* CFBP 2431^T, CFBP 10147, and CFBP 10148 reacted with 11 (65%), 8 (47%), and 11 (65%) of the 17 *P. corrugata* strains tested, respectively. Eight strains produced a positive reaction with one serum, five strains produced a positive reaction with two sera, and four strains produced a positive reaction with three sera. The antisera against strains CFBP 10147 and CFBP 10148 reacted with 8 (53%) and 14 (93%) of the 15 FPTPN strains tested, respectively. It was noted that the serum raised against *P. corrugata* type strain CFBP 2431 did not react with any of the FPTPN strains.

Antisera against FPTPN strains CFBP 10529, CFBP 10530, and CFBP 10569 reacted with 7 (41%), 11 (65%), and 3 (18%) of the 17 *P. corrugata* strains tested, respectively, and with 7 (47%), 12 (80%), and 2 (13%) of the 15 FPTPN strains tested, respectively. Five of the FPTPN strains produced a positive reaction with one serum, five produced a positive reaction with two sera, two produced a positive reaction with three sera, and three did not react with any of the three sera tested. The serum against strain CFBP 10530 reacted with most of the *P. corrugata* (65%) and FPTPN (80%) strains tested, whereas the serum against strain CFBP 10569 reacted only with 19% of the *P. corrugata* strains and with FPTPN strains of DNA hybridization group 3. The serological results did not show a close correlation with the groups obtained from the phenotypic characterization.

LPS patterns. The LPS patterns were compared on the basis of the number, intensity, coloring, and mobility of the bands in silver-stained polyacrylamide gels. *P. corrugata* strains which have been studied previously produced the same patterns which have been described previously (Table 6) (39). Three additional strains of *P. corrugata* (CFBP 10058, CFBP 10436, and CFBP 10558) produced the new LPS profiles shown in Fig.

TABLE 4. Levels of DNA relatedness among *P. corrugata* and FPTPN strains

Source of unlabeled DNA	Phenon or subphenon	% Relative binding at 70°C with labeled DNA from:			
		<i>P. corrugata</i> CFBP 2431 ^T	FPTPN strains		
			CFBP 10529	CFBP 10530	CFBP 10569
<i>P. corrugata</i> strains					
CFBP 2431 ^T	1a	100	49	45	NT ^a
CFBP 10058	1a	106	47	45	NT
CFBP 10146	1a	72	49	45	NT
CFBP 10886	1a	99	44	47	NT
CFBP 10888	1a	91	52	49	NT
CFBP 10896	1a	96	48	52	NT
CFBP 10900	1a	98	51	49	NT
CFBP 10904	1a	99	46	46	NT
CFBP 10905	1a	80	47	54	NT
CFBP 10946	1a	68 (2.0) ^b	48	46	NT
CFBP 10148	1b	69 (3.0)	51	49	NT
CFBP 10558	1b	66 (3.0)	47	50	NT
CFBP 10883	1b	68 (3.5)	47	45	NT
CFBP 10906	1b	96	46	44	NT
CFBP 10436	2a	92	45	40	NT
Avg ± SD		86.7 ± 14.4	47.8 ± 2.3	47.1 ± 3.5	NT
FPTPN strains					
Genomospecies FP1					
CFBP 10552	1a	52	72	54	NT
CFBP 10435	2a	47	88	53	23
CFBP 10529	2a	55	100	58	28
CFBP 10604	2a	53	73	40	NT
CFBP 10531	2b	50	97	48	NT
CFBP 10533	2b	47	89	54	NT
CFBP 10551	2b	51	94	58	NT
CFBP 10567	2b	45	101	50	26
CFBP 10591	2b	51	67 (1.7)	52	NT
Avg ± SD		50.1 ± 3.2	86.8 ± 13.0	51.9 ± 5.5	25.7 ± 2.5
Genomospecies FP2					
CFBP 10437	2a	48	60	89	NT
CFBP 10605	2a	45	57	95	24
CFBP 10934	2a	51	56	94	NT
CFBP 11062	2a	47	55	93	24
CFBP 10530	2b	45	60 (6.0)	100	27
CFBP 10535	ip ^c	50	59	101	NT
Avg ± SD		47.7 ± 2.5	57.8 ± 2.1	95.3 ± 4.3	25.0 ± 1.7
Genomospecies FP3					
CFBP 10524	2c	20	22	18	93
CFBP 10569	2c	23	26	21	100
CFBP 10583	2c	22	23	22	86
Avg ± SD		21.7 ± 1.5	22.7 ± 2.0	20.3 ± 2.1	93.0 ± 7.0

^a NT, not tested.^b The values in parentheses are ΔT_m values (in degrees Celsius).^c ip, isolated phenotype in phenon 2 (at a distance of 0.150).

2. Among the 18 FPTPN strains in phenon 2, 10 different patterns (patterns A to J) were observed (Table 6 and Fig. 2). Ten (66.7%) of the 15 strains belonging to DNA hybridization groups 1 and 2 produced LPS pattern A or B, whereas the three DNA hybridization group 3 strains produced three different profiles (patterns C, D, and J; the last two profiles corresponded to rough type LPS). All of the strains that produced LPS pattern A except strain CFBP 10529 reacted with

the antisera against FPTPN strains CFBP 10529 and CFBP 10530 and with the antisera against *P. corrugata* CFBP 10147 and CFBP 10148. Strains that produced LPS pattern B reacted only with the antisera raised against FPTPN strain CFBP 10530 and against *P. corrugata* CFBP 10148. Strains CFBP 10569 and CFBP 10583, with rough type LPS (patterns D and J), reacted with all of the antisera used except the antiserum prepared against *P. corrugata* type strain CFBP 2431.

TABLE 5. Levels of relatedness between DNAs of *P. corrugata* CFBP 2431^T and FPTPN strains CFBP 10529, CFBP 10530, and CFBP 10569, and DNAs of reference strains of fluorescent and nonfluorescent *Pseudomonas*, *Acidovorax*, *Burkholderia*, *Herbaspirillum*, and *Ralstonia* species

Source of unlabeled DNA	% Relative binding at 70°C with labeled DNA from:			
	<i>P. corrugata</i> CFBP 2431 ^T	FPTPN strains		
		CFBP 10529	CFBP 10530	CFBP 10569
Fluorescent oxidase-positive <i>Pseudomonas</i> strains				
<i>P. aeruginosa</i> CFBP 2466 ^T	15	13	14	10
<i>P. agarici</i> CFBP 2063	18	19	21	13
<i>P. aspleni</i> CFBP 3279	22	23	26	30
<i>P. aureofaciens</i> CFBP 2133 ^T	28	33	36	26
" <i>P. blatchfordae</i> " CFBP 3280 ^T	25	29	32	48
<i>P. cichorii</i> CFBP 2101 ^T	18	26	23	11
<i>P. chlororaphis</i> CFBP 2132 ^T	28	31	29	24
<i>P. flectens</i> CFBP 3281 ^T	2	0	0	0
<i>P. fluorescens</i> CFBP 2102 ^T	24	26	32	42
<i>P. fuscovaginae</i> CFBP 2065 ^T	21	24	22	17
<i>P. marginalis</i> CFBP 1387 ^T	22	28	30	45
<i>P. putida</i> CFBP 2066 ^T	18	12	30	15
<i>P. tolaasii</i> CFBP 2068 ^T	25	27	29	39
Fluorescent oxidase-negative <i>Pseudomonas</i> strains				
<i>P. caricapapayae</i> CFBP 3204 ^T	17	15	19	13
<i>P. savastanoi</i> CFBP 1670 ^T	9	9	11	11
<i>P. syringae</i> pv. <i>syringae</i> CFBP 1392 ^T	10	14	14	12
<i>P. syringae</i> pv. <i>tomato</i> CFBP 2212 ^T	10	14	13	14
<i>P. syringae</i> pv. <i>porri</i> CFBP 1908 ^T	11	12	8	12
Nonfluorescent <i>Pseudomonas</i> strains				
<i>P. alcaligenes</i> CFBP 2437 ^T	11	12	10	2
<i>P. cissicola</i> CFBP 2432 ^T	3	1	2	NT ^a
<i>P. meliae</i> CFBP 3225	0	0	1	NT
<i>P. stutzeri</i> CFBP 2443 ^T	0	0	0	1
<i>P. syzygii</i> CFBP 10974	0	2	1	NT
<i>Acidovorax</i> sp. strains				
<i>A. avenae</i> subsp. <i>avenae</i> CFBP 2425 ^T	1	2	0	1
<i>A. avenae</i> subsp. <i>cattleyae</i> CFBP 2423 ^T	1	2	4	2
<i>A. avenae</i> subsp. <i>citrulli</i> CFBP 10880	1	1	2	NT
<i>A. avenae</i> subsp. <i>konjaci</i> CFBP 10881	1	4	0	NT
<i>Burkholderia</i>, <i>Herbaspirillum</i>, and <i>Ralstonia</i> spp. strains				
<i>B. andropogonis</i> CFBP 2421 ^T	0	5	0	1
<i>B. caryophylli</i> CFBP 2429 ^T	3	2	0	NT
<i>B. cepacia</i> CFBP 2227 ^T	1	1	1	2
<i>B. gladioli</i> CFBP 2427 ^T	3	2	0	NT
<i>B. glumae</i> CFBP 2430 ^T	3	2	0	NT
<i>B. plantarii</i> CFBP 3199	1	4	0	NT
<i>H. rubrisubalbicans</i> CFBP 1202 ^T	1	1	0	NT
<i>R. solanacearum</i> CFBP 2047 ^T	2	1	3	1

^a NT, not tested.

FAME profiles. Table 7 shows the fatty acids used in this study. Other fatty acid peaks appeared occasionally in the chromatographic profiles as traces and were not considered for analysis in this study. Numerical analysis of the FAME profiles allowed us to define four main clusters and three isolated strains (Fig. 3). A comparison between the DNA hybridization groups described above and the FAME clusters indicated that cluster I contains only *P. corrugata* strains, which can be differentiated mainly on the basis of four fatty acids (14:0 3OH, 16:0 3OH, unknown 13961, and unknown 14503), and that cluster II contains strains belonging to different DNA hybridization groups (*P. corrugata* and FPTPN strains belonging to DNA hybridization groups 1 and 2). The three DNA hybridization group 3 strains were included in cluster III. Cluster IV contained three DNA hybridization group 1 and 2 FPTPN

strains, some of which had a profile with the characteristic fatty acids used to differentiate *P. corrugata*. Table 7 shows the mean percentages of fatty acids in the four different clusters.

Pathogenicity tests. In the first experiment, 28 (93%) of the 30 *P. corrugata* strains tested and 13 (93%) of the 14 FPTPN strains belonging to DNA hybridization groups 1 and 2 tested produced typical symptoms (i.e., necrosis and pith hollowing) on at least one of the five inoculated tomato plants. The development of external symptoms (browning of stem surface and adventitious roots) and the length of pith necrosis were variable according to strain. In contrast, none of the three FPTPN strains belonging to DNA hybridization group 3 (strains CFBP 10524, CFBP 10569, and CFBP 10583) produced typical symptoms of pith necrosis. No symptoms were observed in control plants.

TABLE 6. ELISA-I reactivities and LPS patterns of *P. corrugata* and FPTPN strains

Strain	Phenon or subphenon	Reactivity in ELISA-I with antiserum against:						LPS pattern ^b
		<i>P. corrugata</i> strains			FPTPN strains			
		CFBP 2431 ^T	CFBP 10147	CFBP 10148	CFBP 10529	CFBP 10530	CFBP 10569	
<i>P. corrugata</i> strains								
CFBP 2431 ^T	1a	+	—	—	—	+	—	1
CFBP 10058	1a	—	+	+	+	+	—	NP
CFBP 10060	1a	+	—	—	—	—	—	1
CFBP 10145	1a	+	+	+	—	+	—	2
CFBP 10147	1a	+	+	—	—	+	—	ND
CFBP 10148	1b	—	+	+	+	+	—	3
CFBP 10436	2a	—	—	+	—	—	—	NP
CFBP 10553	1a	+	—	+	—	—	—	ND
CFBP 10558	1b	—	+	+	+	+	+	NP
CFBP 10883	1b	+	+	+	+	+	+	6
CFBP 10886	1a	—	—	+	—	+	—	4
CFBP 10888	1a	+	—	—	—	—	—	1
CFBP 10896	1a	—	—	+	+	+	—	11
CFBP 10900	1a	+	+	+	+	+	—	14
CFBP 10904	1a	+	—	—	—	—	—	5
CFBP 10905	1a	+	—	—	—	—	—	1
CFBP 10906	1b	+	+	+	+	+	+	3
FPTPN strains								
Genomospecies FP1								
CFBP 10435	2a	—	—	+	—	—	—	G
CFBP 10529	2a	—	—	—	+	+	—	A
CFBP 10531	2b	—	—	—	—	+	—	B
CFBP 10533	2b	NT	NT	NT	NT	NT	NT	B
CFBP 10551	2b	—	—	+	—	+	—	B
CFBP 10552	1b	—	+	+	+	+	—	A
CFBP 10567	2b	—	—	+	—	+	—	B
CFBP 10591	2b	—	+	+	—	—	—	E
CFBP 10604	2a	—	+	+	—	+	—	F
Genomospecies FP2								
CFBP 10437	2a	—	+	+	+	+	—	A
CFBP 10530	2b	—	—	+	—	+	—	B
CFBP 10535	2	NT	NT	NT	NT	NT	NT	H
CFBP 10605	2a	—	+	+	+	+	—	A
CFBP 10934	2a	NT	NT	NT	NT	NT	NT	I
CFBP 11062	2a	—	+	+	+	+	—	A
Genomospecies FP3								
CFBP 10524	2c	—	—	+	—	—	—	C
CFBP 10569	2c	—	+	+	+	+	+	D
CFBP 10583	2c	—	+	+	+	+	+	J

^a +, positive; —, negative; NT, not tested. A reaction was considered positive when the absorbance was twice the absorbance of the negative control in each plate.

^b ND, not determined; NP, new LPS profile for *P. corrugata* (see Fig. 2). The numbers are designations for LPS profiles previously described by Siverio et al. (39), and the letters are designations for LPS profiles determined for FPTPN strains (Fig. 2).

The results of the second experiment, in which we inoculated two strains of *P. corrugata* and four FPTPN strains into pepper, tobacco, bean, cauliflower, melon, cucumber, and zucchini plants, are shown in Table 8. No symptoms were observed in any of the control plants except some melon and cucumber plants which, for unknown reasons, showed slight symptoms of pith necrosis. Nevertheless, no pseudomonads were isolated from the lesions. *P. corrugata* and FPTPN strains were able to cause pith necrosis in pepper, tobacco, bean, cauliflower, melon, and cucumber plants. No symptoms were observed in zucchini plants. A great deal of variability was observed in the length of the necrosis in the plants inoculated with each strain of *P. corrugata*, as well as with FPTPN strains. The pith necrosis obtained differed slightly in different hosts in color, intensity, and extension to other tissues. Colonies with the charac-

teristics of *P. corrugata* or of fluorescent strains were reisolated from the edges of the lesions.

DISCUSSION

In this study, we compared a collection of 33 FPTPN strains with 89 *P. corrugata* strains and with 74 reference strains of fluorescent and nonfluorescent *Pseudomonas*, *Acidovorax*, *Burkholderia*, *Herbaspirillum*, and *Ralstonia* species.

A numerical analysis of the results obtained in 167 biochemical and physiological tests was performed. Eighty-eight of the 89 *P. corrugata* strains, including type strain CFBP 2431, formed a single cluster (phenon 1). These strains are nonfluorescent on King B medium, do not produce levan, are oxidase positive and nonpectolytic, reduce nitrates, and accumulate

FIG. 2. Polyacrylamide gel electrophoresis of whole-cell lysates treated with proteinase K, showing the different LPS patterns. The *P. corrugata* strains used were CFBP 2431^T, CFBP 10058, CFBP 10436, and CFBP 10558, which had a profile without an O-chain (rough type LPS). The following FFTP strains were used; lane A, CFBP 10529; lane B, CFBP 10533; lane C, CFBP 10524; lane D, CFBP 10569 (rough type LPS); lane E, CFBP 10591; lane F, CFBP 10604; lane G, CFBP 10435; lane H, CFBP 10535; and lane I, CFBP 10934. The letters correspond to the LPS patterns in Table 6.

PHB, as previously described (25, 35, 39). The results of previous studies performed with a limited number of strains are contradictory with regard to arginine dihydrolase activity in *P. corrugata* and the ability of this organism to induce a hypersensitive reaction on tobacco leaves (19, 25, 35, 39). Our results obtained with a large number of strains indicated that 77% of the *P. corrugata* strains tested are positive for arginine dihydrolase activity, provided that a long incubation time (at least 15 days) is used, and that only 19% of the *P. corrugata* strains tested produce a hypersensitivity reaction on tobacco leaves. Thus, the results of these tests are variable for this species. These characteristics correspond approximately to the characteristics of group Va in the LOPAT determinative scheme (24), which also includes saprophytic and pathogenic fluorescent *Pseudomonas* species, such as *P. fluorescens*, *P. putida*, and *P. aeruginosa*.

Considering the phenotypic distances between the different clusters, *P. corrugata* appeared to be very closely related to FFTP strains and more closely related to fluorescent *Pseudomonas* species than to nonfluorescent *Pseudomonas*, *Acidovorax*, *Herbaspirillum*, and *Burkholderia* species. Nevertheless, *P. corrugata* can be clearly distinguished by many phenotypic characteristics from the major plant-pathogenic or saprophytic, fluorescent, oxidase-positive *Pseudomonas* species, such as *P. cichorii*, *P. fuscovaginae*, *P. tolaasii*, *P. marginalis*, *P. fluorescens*, and *P. putida*. Our phenotypic results, obtained with a large number of strains and with numerous tests, are very informative and should be useful for precisely defining the species *P. corrugata*. Our phenotypic observations are strengthened by our DNA-DNA reassociation results; the levels of hybridization obtained with labeled DNA from *P. corrugata* type strain CFBP 2431 were as high as 55% with FFTP strains and varied from 15 to 28% with the type strains of fluorescent *Pseudomonas* species, but were generally less than 5% with the type strains of nonfluorescent *Pseudomonas*, *Acidovorax*, *Burkholderia*, *Herbaspirillum*, and *Ralstonia* species.

TABLE 7. Fatty acid compositions of the clusters obtained by numerical analysis^a

FAME ^b	Fatty acid compositions							
	Cluster I (n = 7) ^c		Cluster II (n = 17)		Cluster III (n = 3)		Cluster IV (n = 3)	
	Mean % (SD)	No. of strains with fatty acid	Mean % (SD)	No. of strains with fatty acid	Mean % (SD)	No. of strains with fatty acid	Mean % (SD)	No. of strains with fatty acid
10:0	0.42 (0.11)	7	0.18 (0.06)	17	0.06 (0.02)	3	0.32 (0.07)	3
10:0 3OH	4.28 (0.28)	7	3.68 (0.31)	17	3.14 (0.35)	3	3.76 (0.19)	3
12:0	3.59 (0.51)	7	5.23 (0.70)	17	2.90 (0.04)	3	3.93 (0.49)	3
Unknown 12486	0.21 (0.05)	7	0.09 (0.07)	13	0.07 (0.01)	3	0.42 (0.13)	3
12:0 2OH	3.92 (0.49)	7	3.10 (0.48)	17	4.49 (0.08)	3	3.94 (0.33)	3
12:1 3OH	0.79 (0.24)	7	0.09 (0.08)	12	0.01 (0.01)	1	0.74 (0.19)	3
12:0 3OH	4.52 (0.29)	7	4.15 (0.26)	17	4.30 (0.03)	3	4.39 (0.33)	3
Unknown 13961	0.84 (0.21)	7	0.00 (0.00)	0	0.00 (0.00)	0	0.08 (0.12)	1
14:0	0.06 (0.08)	3	0.35 (0.07)	17	0.47 (0.06)	3	0.30 (0.05)	3
Unknown 14503	0.63 (0.21)	7	0.00 (0.00)	0	0.00 (0.00)	0	0.15 (0.14)	2
15:0	0.02 (0.03)	2	0.02 (0.04)	5	0.07 (0.01)	3	0.17 (0.01)	3
14:0 3OH	0.98 (0.23)	7	0.00 (0.02)	1	0.00 (0.00)	0	0.72 (0.51)	3
16:1 cis 9	30.23 (1.44)	7	35.63 (1.61)	17	31.02 (0.08)	3	33.14 (2.22)	3
16:0	26.42 (0.72)	7	27.21 (1.99)	17	29.19 (1.57)	3	24.88 (0.75)	3
17:0 cyclo	2.91 (0.65)	7	0.90 (0.73)	15	2.16 (0.73)	3	2.39 (0.48)	3
17:0	0.01 (0.03)	1	0.06 (0.07)	8	0.14 (0.04)	3	0.29 (0.04)	3
16:0 3OH	0.60 (0.16)	7	0.00 (0.00)	0	0.00 (0.00)	0	0.22 (0.31)	1
18:1 cis 11	18.60 (0.84)	7	18.71 (2.42)	17	20.89 (2.33)	3	19.31 (0.40)	3
18:0	0.67 (0.08)	7	0.55 (0.16)	17	0.89 (0.07)	3	0.73 (0.22)	3
19:0 cyclo 11-12	0.30 (0.13)	7	0.04 (0.10)	4	0.20 (0.05)	3	0.12 (0.08)	2

^a Clusters as shown in the fatty acid dendrogram in Fig. 3.

^b The numbers in the unknown FAME designations are the equivalent chain lengths.

^c The numbers in parentheses are the numbers of strains in the clusters.

FIG. 3. Dendrogram based on the results of the fatty acid cluster analysis, showing the relationships among 33 *Pseudomonas* strains isolated from tomato and pepper with pith necrosis. Abbreviations: Pc, *P. corrugata*; 1, *Pseudomonas* genomospecies FP1; 2, *Pseudomonas* genomospecies FP2; 3, *Pseudomonas* genomospecies FP3.

By using DNA-rRNA hybridization, it has been demonstrated that *P. corrugata* belongs to superfamily 2 and to the *P. fluorescens* rRNA branch now considered the authentic pseudomonads (11, 12). However, as a nonfluorescent species, *P. corrugata*, like *Pseudomonas amygdali*, could be considered anomalous in this rRNA branch (48). Moreover, chemotaxonomic studies based on analyses of quinones and fatty acids have placed *P. corrugata* in the same fluorescent *Pseudomonas* group (42).

In the present study, *P. corrugata* phenon 1 was divided into two subphenon, subphenon 1a and 1b, which were differentiated by the following three biochemical tests: assimilation of meso-tartrate, isobutyrate, and histamine. We did not observe any correlation between the geographic origin of the *P. corrugata* strains and their distribution in subphenon 1a and 1b. No correlation was found between the subdivision of phenon 1 into subphenon 1a and 1b on the basis of phenotypic characteristics and the results of DNA-DNA hybridizations. The accepted molecular definition of a bacterial genomic species (genomo-

species) is applied to strains whose levels of DNA-DNA hybridization are around 70%, with ΔT_m values of 5°C or less (46). Our DNA-DNA hybridization results obtained with labeled DNA from *P. corrugata* type strain CFBP 2431 indicated clearly that *P. corrugata* strains belong to a single genomic group and confirmed that the data for *P. corrugata* are consistent with the definition of a bacterial species (46). The G+C content of type strain CFBP 2431^T (59.6 mol%) was similar to the values previously reported for *P. corrugata* (11). However, the range of levels of DNA reassociation (66 to 106%) provides evidence of genomic variability in the *P. corrugata* strains tested here. These results could be related to the long evolution of a weakly pathogenic bacterium. Nevertheless, as the genomic diversity is not strictly correlated with phenotypic clustering, we do not propose that *P. corrugata* should be split into subspecies. However, in our opinion, it would be very interesting to further study the genomic diversity of *P. corrugata* at the infraspecific level by molecular techniques, such as

TABLE 8. Pathogenicity of *P. corrugata* and FPTPN strains for different host plants

Strain	Pathogenicities for the following host plants:						
	Pepper	Tobacco	Bean	Cauliflower	Melon	Cucumber	Zucchini
<i>P. corrugata</i> strains							
CFBP 2431 ^T	+	++	++	++	+	+	-
CFBP 10148	++	+	+	+	-	+	-
FPTPN strains							
CFBP 10529 (genomospecies FP1)	+	++	++	++	-	-	-
CFBP 10530 (genomospecies FP2)	+	+	++	+	-	+	-
CFBP 10934 (genomospecies FP2)	+	++	+	++	-	+	-
CFBP 10583 (genomospecies FP3)	-	-	+	++	+	+	-

^a -, no internal or external lesions observed; +, pith necrosis 10 to 20 mm long, ++, pith necrosis more than 20 mm long.

restriction fragment length polymorphism or randomly amplified polymorphic DNA analysis.

Seventeen of the 33 FPTPN strains belonged to phenon 2, and one (strain CFBP 10552) belonged to *P. corrugata* phenon 1. The strains in phenon 2 can be distinguished from *P. corrugata* strains by the following four biochemical characteristics: production of fluorescent pigment on King B medium, accumulation of PHB, levan formation, and assimilation of sorbitol. Moreover, phenon 2 contained one *P. corrugata* strain (CFBP 10436), and, conversely, *P. corrugata* phenon 1 contained one FPTPN strain (CFBP 10552). Consequently, these two phenon are closely related phenotypically. This could be explained by phytopathological specialization, as the organisms produced gave similar symptoms on the same host plant, tomato. However, DNA-DNA hybridizations revealed that these 18 FPTPN strains are clearly separate from *P. corrugata* and belong to three different DNA hybridization groups that could be proposed as new species (46). The G+C contents of these DNA hybridization groups, which varied from 59.2 to 60.2 mol%, fall within the range of G+C contents of the genus *Pseudomonas* (55.2 to 67.2 mol%) (11, 31). As suggested by Johnson (20) in a discussion of the significance of levels of DNA-DNA hybridization between genomic species, we compared the levels of DNA-DNA hybridization between *P. corrugata* and FPTPN strains; these values indicated that DNA hybridization groups 1 and 2 are closely related (levels of hybridization, 51.9 to 57.8%) to each other and are related to *P. corrugata* (levels of hybridization, 47.1 to 47.8%), whereas DNA hybridization group 3 is less closely related to DNA hybridization groups 1 and 2 (levels of hybridization, 20.3 to 25.7%) and to *P. corrugata* (levels of hybridization, 20 to 23%). The strains of DNA hybridization groups 1 and 2 are distributed at random in subphenon 2a and 2b and cannot be clearly distinguished by phenotypic tests. DNA hybridization group 3, containing only three strains, can be distinguished from DNA hybridization groups 1 and 2 by only two biochemical tests (assimilation of citraconate and laevulinate) and by the absence of virulence on tomato plants under our experimental conditions. Because of the lack or insufficiency of discriminating phenotypic tests and the low number of strains in DNA hybridization group 3, DNA hybridization groups 1, 2, and 3 are designated *Pseudomonas* genomospecies FP1, FP2, and FP3 (FP for fluorescent *Pseudomonas*), respectively, rather than given formal names and proposed as new species.

The involvement of fluorescent *Pseudomonas* spp. strains in tomato pith necrosis has been described previously in different countries (3, 6, 13). Strains CFBP 10435 and CFBP 10437, isolated in Canada and previously described as a novel agent of tomato stem necrosis (13), belong to *Pseudomonas* genomospecies FP1 and FP2, respectively.

Of the remaining 15 FPTPN strains which did not cluster in phenon 2, 2 strains clustered in phenon 12, which is closely related to *P. cichorii* (phenon 11); these two strains correspond to group III of Leliott et al. (24) and could be considered *P. cichorii* strains. Five strains which clustered in *P. marginalis* (phenon 5), two of which (CFBP 10539 and CFBP 10549) were pectolytic, were identified as strains of *P. marginalis* (group IVa of Leliott et al. [24]). One strain (CFBP 10582) was included with *P. tolaasii* in phenon 6 but does not have typical phenotypic characteristics of *P. tolaasii* (36). Two strains (CFBP 10601 and CFBP 10602) clustered in phenon 8 and were identified as *P. aeruginosa* strains. It was impossible to identify the last five strains, which were isolated phenotypes. To our knowledge, among the well-characterized fluorescent phytopathogenic *Pseudomonas* species, only *P. cichorii* has been described as a causal agent of tomato pith necrosis in New Zealand (47).

However, the involvement of pectolytic *P. marginalis* strains in this disease cannot be definitively ruled out. No clear conclusion concerning the strains related to saprophytic fluorescent *Pseudomonas* species, such as *P. fluorescens* and *P. aeruginosa*, can be reached in the absence of results concerning the pathogenicity of these strains on tomato plants.

The LPS characteristics of *P. corrugata* and FPTPN strains were investigated by using a procedure that facilitates the differentiation of these strains. However, the same LPS profiles may occasionally have different compositions (39), and it is necessary to use biochemical or serological techniques to verify them. New LPS profiles, different from those previously described (39), were observed in the *P. corrugata* strains studied. In FPTPN strains, original LPS profiles that were different from those of *P. corrugata* strains (39; this study) were observed. Our results show that the genomospecies delineated by DNA-DNA hybridization include strains which have different LPS profiles, indicating that there is LPS heterogeneity within each genomospecies. However, some LPS profiles coincide with different genomospecies, particularly genomospecies FP1 and FP2. Therefore, although different at the genomic level, strains of these two genomospecies can have LPS structures in common. Strains of genomospecies FP3 have LPS profiles different from those of genomospecies FP1 and FP2. This observation, together with the results of numerical taxonomy and DNA-DNA hybridization analyses, indicate that genomospecies FP3 has a low-level relationship with genomospecies FP1 and FP2. The serological variability of *P. corrugata* strains has been investigated previously (39) and is attributed mainly to variations in the composition of LPS. *P. corrugata* and FPTPN strains were serotyped by performing an ELISA-I with antisera raised against strains belonging to these two groups. A high level of serological variability was observed in the strains. The reactivities of *P. corrugata* strains with the three anti-*P. corrugata* sera tested and the reactivities of FPTPN strains with the three sera raised against FPTPN strains were very variable; a similar phenomenon has been reported previously for *P. corrugata* isolates from Spain and Italy (6, 39). Moreover, cross-reactions (i.e., reactivity of anti-*P. corrugata* sera with FPTPN strains and reactivity of sera against FPTPN strains with *P. corrugata*) were observed. These cross-reactions indicate that *P. corrugata* and FPTPN strains share common epitopes in spite of their heterogeneity. For FPTPN strains, serological reactions seem to be directly related to LPS profiles, since strains with the same LPS profile reacted with the same antisera, as previously observed for *P. corrugata* (39).

Numerical analysis of FAME profiles revealed that most (about 80%) of the *P. corrugata* strains studied were distributed in two clusters, clusters I and II. Cluster I plus strain CFBP 10060, which is closely attached to this cluster, had a characteristic *P. corrugata* profile with four fatty acids (14:0 3OH, 16:0 3OH, unknown 13961, and unknown 14503), as previously described (42), whereas the strains in cluster II had a different profile. This difference might be associated with changes in the morphological aspect of colonies, as previously reported for this species (40) and for *Burkholderia cepacia* and *Burkholderia gladioli* (43). Although this phenomenon was not investigated here, this hypothesis is supported by the fact that cluster II strains had a profile with peak areas within the range described for *P. corrugata* strains along with differences in colony morphology (40). Strains belonging to genomospecies FP1 and FP2 overlap in the peak areas of their profiles in cluster II (along with six *P. corrugata* strains) and cluster IV (sometimes with characteristic peaks of *P. corrugata*). Consequently, the FAME analysis did not permit differentiation of genomospecies FP1 and FP2, which had profiles similar to the

profiles of other species, such as *P. cichorii*, *Pseudomonas viridiflava*, and *Pseudomonas syringae* (42) (first choices with a high level of similarity when the MIDI identification system was used). The three strains of genomospecies FP3 had a low degree of variation in peak area percentages, but they had a profile similar to the profiles of species such as *P. marginalis*, *P. fluorescens*, and *P. putida* (42) (first choices with a high level of similarity when the MIDI identification system was used).

Inoculation of tomato plants in the greenhouse indicated that a large majority of the *P. corrugata* strains (93%) produced typical symptoms, mainly pith necrosis and hollowing, as previously reported (25, 35, 39). Most (93%) of the FPTPN strains belonging to genomospecies FP1 and FP2 also produced these typical symptoms. The intensity and the extent of the symptoms varied a great deal with the strain for both *P. corrugata* and FPTPN strains. Strains of genomospecies FP3 did not produce any symptoms, suggesting either that they are non-pathogenic or that the conditions which we used were not favorable for these strains. Both *P. corrugata* and FPTPN strains induced pith necrosis symptoms in plants other than tomato (i.e., pepper, tobacco, bean, cauliflower, melon, and cucumber) but not in zucchini. The symptoms in these different hosts were similar to those reported by Siverio (38), but in that previous study, symptoms in zucchini were also observed. In the present study, the number of strains tested was limited, and therefore, no definitive conclusion can be drawn concerning the host ranges of *P. corrugata* and FPTPN strains. Although natural infections have been observed in a limited number of host plants (tomato, pepper, and chrysanthemum) (14, 25, 35), our results suggest that these bacteria have a broad pathogenic potential, which is a characteristic of opportunistic organisms. It can be supposed that under favorable conditions, *P. corrugata* and FPTPN strains can cause symptoms not only on tomato, but also on many cultivated plants of different species.

Thus, we confirmed that tomato pith necrosis can be due to different *Pseudomonas* species (mainly *P. corrugata*, but also fluorescent oxidase-positive *Pseudomonas* species), as previously reported (3, 6, 13). On the basis of DNA-DNA hybridization, physiological, and biochemical characteristics, serological data, LPS patterns, FAME profiles, and pathogenicity test data, below we provide an emended description of *P. corrugata* and descriptions of three new fluorescent *Pseudomonas* genomospecies, designated genomospecies FP1, FP2, and FP3.

Emended description of *Pseudomonas corrugata* Roberts and Scarlett 1981. *P. corrugata* is a gram-negative, non-spore-forming rod. Colonies on YBGA are wrinkled or smooth and frequently produce yellow to brown pigments. Mobile cells have multiple polar flagella, as previously reported (35). This organism is strictly aerobic, nonfluorescent on King B medium, and oxidase positive, does not produce levan, is not pectolytic, reduces nitrates into nitrites, and accumulates PHB as previously described (25, 35, 39). Most (about 80%) of the strains are arginine dihydrolase positive after 15 days of incubation, and a few (about 20%) of the strains produce hypersensitivity on tobacco leaves. Hydrolysis of Tween 80 and hydrolysis of gelatin are variable.

P. corrugata strains utilize acetate, *N*-acetylglucosamine, aconitate, D- and L-alanine, DL-4-aminobutyrate, DL-5-aminovalerate, D-arabitol, L-arabinose, L-arginine, L-aspartate, betaine, caprate, *n*-caproate, caprylate, citrate, diaminobutane, D-fructose, fumarate, D-galactose, glucosamine, D-glucose, gluconate, L-glutamate, glutarate, glycerol, heptanoate, L-histidine, DL-3-hydroxybutyrate, *p*-hydroxybenzoate, inositol, L-isoleucine, 2-ketoglutarate, DL-lactate, L-leucine, L-malate, malonate, mannitol, D-mannose, pelargonate, L-phenylalanine, L-proline, propionate, ribose, sarcosine, L-serine, spermine,

saccharose, succinate, threolose, trigonelline, L-tyrosine, *n*-valerate, L-valine, and D-xylose.

In contrast, they do not hydrolyze starch, DNA, and esculin and do not utilize acetamide, adipate, adonitol, 2-, 3-, and 4-aminobenzoate, DL-2-aminobutyrate, amygdalin, D-arabinose, L-arabitol, arbutin, azelate, benzoate, benzylamine, butylamine, D-cellobiose, L-citrulline, creatine, L-cysteine, dulcitol, ethylamine, erythritol, D- and L-fucose, D-gentobiose, glycine, glycogen, glycolate, *o*- and *m*-hydroxybenzoate, inulin, itaconate, 5-ketogluconate, DL-kynurenine, lactose, laevulinate, maleate, maltose, D- and L-mandelate, D-melezitose, D-melibiose, mesaconate, L-methionine, methyl-D-glucoside, methyl-D-mannoside, methylxyloside, DL-norvaline, oxalate, phenylacetate, phthalate, isophthalate, *ter*-phthalate, pimelate, D-raffinose, rhamnose, salicin, sebacate, sorbitol, suberate, L-tartrate, D-tagatose, L-threonine, tryptamine, D- and L-tryptophan, D-turanose, urea, xylitol, and L-xylose.

Utilization of the following substrates is variable: DL-3-aminobutyrate, amylamine, butyrate, isobutyrate, citraconate, ethanolamine, DL-glycerate, histamine, 2-ketogluconate, L-lysine, D-lyxose, D-malate, L-norleucine, L-ornithine, isovalerate, D-tartrate, *meso*-tartrate, and pyruvate.

The levels of DNA relatedness of *P. corrugata* strains with type strain CFBP 2431 range from 66 to 99% (as determined by the S1 nuclease-trichloroacetic acid method). The G+C content of the DNA is 58.4 to 60.8 mol% (11; this study).

Description of *Pseudomonas* genomospecies FP1, FP2, and FP3. *Pseudomonas* genomospecies FP1, FP2, and FP3 are gram-negative rods which are mobile by means of multiple polar flagella. The strains are oxidase positive and strictly aerobic, produce fluorescent pigment on King B medium, do not accumulate PHB, are arginine dihydrolase positive, and reduce nitrates into nitrites. They are not pectolytic and do not induce hypersensitivity on tobacco leaves.

Strains of *Pseudomonas* genomospecies FP1, FP2, and FP3 utilize ethanolamine, DL-glycerate, isobutyrate, isovalerate, 2-ketogluconate, D-lyxose, and D-malate. Utilization of saccharose, succinate, trigonelline, and D-xylose is variable.

Strains of *Pseudomonas* genomospecies FP1 and FP2, which cannot be distinguished phenotypically, produce levan and utilize sorbitol but not citraconate and laevulinate. Hydrolysis of gelatin and Tween 80 and assimilation of itaconate, *N*-acetylglucosamine, amylamine, and glycine are variable.

Strains of *Pseudomonas* genomospecies FP3 hydrolyze gelatin but not Tween 80 and utilize citraconate, laevulinate, and *N*-acetylglucosamine but not itaconate, amylamine, and glycine. Production of levan and utilization of sorbitol are variable.

Assimilation of the other organic substrates tested is the same for strains of *Pseudomonas* genomospecies FP1, FP2, and FP3 as for *P. corrugata* strains (see above).

The FAME profiles of strains of *Pseudomonas* genomospecies FP1 and FP3 are similar to those of *P. cichorii*, *P. viridiflava*, and *P. syringae* strains. The FAME profiles of strains of *Pseudomonas* genomospecies FP3 are similar to those of *P. fluorescens* and *P. putida* strains. The LPS patterns on silver-stained polyacrylamide electrophoresis gels of strains of *Pseudomonas* genomospecies FP1, FP2, and FP3 are different from those of *P. corrugata* strains. Strains of *Pseudomonas* genomospecies FP1 and FP2 have the same LPS patterns, whereas strains of *Pseudomonas* genomospecies FP3 have LPS patterns that are different from those of strains of *Pseudomonas* genomospecies FP1 and FP2.

Pseudomonas genomospecies FP1 and FP2 are related to each other (levels of DNA relatedness, 40 to 60%) but are weakly related to *Pseudomonas* genomospecies FP3 (levels of

DNA relatedness, 18 to 26% [as determined by the S1 nuclease-trichloroacetic acid method]).

The DNA G+C content is 59.2 mol% for *Pseudomonas* genomospecies FP1 and FP2 and 60.2 mol% for *Pseudomonas* genomospecies FP3 (as determined by the thermal denaturation method). The type strains are strain CFBP 10529 for *Pseudomonas* genomospecies FP1, strain CFBP 10530 for *Pseudomonas* genomospecies FP2, and strain CFBP 10569 for *Pseudomonas* genomospecies FP3. These three type strains were isolated from tomatoes with symptoms of pith necrosis in France from 1980 to 1983. The type strains and additional strains have been deposited in the Collection Française de Bactéries Phytopathogènes (CFBP), Angers, France.

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