Effect of Two Plant Species, Flax (*Linum usitatissimum* L.) and Tomato (*Lycopersicon esculentum* Mill.), on the Diversity of Soilborne Populations of Fluorescent Pseudomonads.

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Suppression of soilborne disease by fluorescent pseudomonads may be inconsistent. Inefficient root colonization by the introduced bacteria is often responsible for this inconsistency. To better understand the bacterial traits involved in root colonization, the effect of two plant species, flax (*Linum usitatissimum* L.) and tomato (*Lycopersicon esculentum* Mill.), on the diversity of soilborne populations was assessed. Fluorescent pseudomonads were isolated from an uncultivated soil and from rhizosphere, rhizoplane, and root tissue of flax and tomato cultivated in the same soil. Species and biovars were identified by classical biochemical and physiological tests. The ability of bacterial isolates to assimilate 147 different organic compounds and to show three different enzyme activities was assessed to determine their intraspecific phenotypic diversity. Numerical analysis of these characteristics allowed the clustering of isolates showing a high level (87.8%) of similarity. On the whole, the populations isolated from soil were different from those isolated from plants with respect to their phenotypic characteristics. The difference in bacteria isolated from uncultivated soil and from root tissue of flax was particularly marked. The intensity of plant selection was more strongly expressed with flax than with tomato plants. The selection was, at least partly, plant specific. The use of 10 different substrates allowed us to discriminate between flax and tomato isolates. *Pseudomonas fluorescens* biovars II, III, and V and *Pseudomonas putida* biovar A and intermediate type were well distributed among the isolates from soil, rhizosphere, and rhizoplane. Most isolates from root tissue of flax and tomato belonged to *P. putida* bv. A and to *P. fluorescens* bv. II, respectively. Phenotypic characterization of bacterial isolates was well correlated with genotypic characterization based on repetitive extragenic palindromic PCR fingerprinting.

Fluorescent pseudomonads can suppress various soilborne diseases (50). They are implicated in the natural suppressiveness of soils toward fusarium wilts (19, 39). These bacteria have been used successfully to control fusarium wilts of various plant species grown in conducive soils and potting media (24). However, the suppression achieved with inoculants is not as effective as with natural soil (40). Overall, biological control of soilborne disease achieved by fluorescent pseudomonads is often inconsistent (24, 50). This inconsistency has been partially associated with inefficient root colonization by the introduced bacteria (41, 50). Indeed, Bull et al. (3) established a linear relationship between population sizes of *P. fluorescens* 2-79 and suppression of take-all.

Among factors affecting bacterial colonization of roots, the plant is postulated to play a major role (13). Plant roots liberate organic compounds to the soil, a process called rhizodeposition (51). The exudation of these compounds is responsible for the rhizosphere effect (26). As early as 1965, Rovira (36) suggested that root exudates play a key role in the selective stimulation of microorganisms. The effect of specific compounds on the selection of rhizospheric microflora was first demonstrated by Gunner et al. (15), who showed that the dominant bacteria in the rhizosphere of bean plants sprayed

with an organophosphate insecticide were those able to use the insecticide metabolites as a nutrient source. Since then, other studies have reported the effect of plants on soilborne microflora (1, 20, 27, 29) or on introduced strains of fluorescent pseudomonads (16, 49). However, information is lacking about the characteristics of soilborne fluorescent pseudomonads selected by the plant (14, 46). The aim of the present study was to determine some bacterial traits related to the selection exerted by the plant toward fluorescent pseudomonads. The diversity of fluorescent pseudomonads in soil and plant-associated habitats with two different plant species was compared. Taxonomic and phenotypic characteristics of fluorescent pseudomonads isolated from an uncultivated soil and from plants cultivated in the same soil were compared. Since the size and composition of the rhizospheric microflora have been described as being plant dependent (4, 29, 37), fluorescent pseudomonads were isolated from two different plant species: *Linum usitatissimum* L. and *Lycopersicon esculentum* Mill.

MATERIALS AND METHODS

Bacterial strains. Two collections of strains were analyzed. The first collection consisted of 30 type and reference strains belonging to the main fluorescent *Pseudomonas* species and to some nonfluorescent *Pseudomonas* species (Table 1). The strains were obtained from the French collection of phytopathogenic bacteria, Institut National de la Recherche Agronomique, Angers, France; the National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom; and the American Type Culture Collection, Rockville, Md. The second collection included wild-type isolates from either an uncultivated silty-loamy soil or

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TABLE 1. Type and reference strains of *Pseudomonas* spp. used in this study

Species and biovar	Strain ^a	Origin
<i>P. aureofaciens</i>	CFBP 2133 ^T (ATCC 13985)	River clay
	CFBP 3155 (CIP 7523)	Unknown
<i>P. chlororaphis</i>	CFBP 2132 ^T (ATCC 9446)	Plate contaminant
<i>P. cichorii</i>	CFBP 2101 ^T (ATCC 10857)	<i>Cichorium endiva</i> , Germany
<i>P. fluorescens</i> bv. I-1	CFBP 2102 ^T (ATCC 13525)	Water, United Kingdom
	CFBP 2123 (ATCC 17397)	Water, Netherlands
<i>P. fluorescens</i> bv. II-1	CFBP 2125 (ATCC 17482)	Water by naphthalene enrichment
	FPFS 638	Soil, France
<i>P. fluorescens</i> bv. III-1	CFBP 2127 (ATCC 17400)	Hen egg, California
	CFBP 3149 (ATCC 17559)	Unknown
<i>P. fluorescens</i> bv. IV-1	CFBP 2129 (ATCC 17513)	Water by hydrocarbon enrichment, California
<i>P. fluorescens</i> bv. IV	CFBP 3150 (ATCC 12983)	Soil
<i>P. fluorescens</i> bv. V	CFBP 2130 (ATCC 17386)	Soil
	CFBP 3151 (ATCC 17562)	<i>Spatum</i> spp.
<i>P. marginalis</i>	CFBP 3153 (ATCC 17819)	Unknown
<i>P. palleronii</i>	CFBP 2445 ^T (ATCC 17724)	Water
<i>P. putida</i> bv. A	CFBP 2066 ^T (ATCC 12633)	Soil by lactate enrichment, United States
	CFBP 3142-1 (ATCC 17430)	Soil
	CFBP 3143-2 (ATCC 17487)	Soil
<i>P. putida</i> bv. B	ATCC 17484	Soil by naphthalene enrichment, United Kingdom
	CFBP 3140-1 (ATCC 17430)	Soil
<i>P. saccharophila</i>	CFBP 2433 ^T (ATCC 15946)	Water
<i>P. setariae</i>	NCPPB 1392 ^T	<i>Oryza sativa</i> , Japan
<i>P. solanacearum</i>	CFBP 2047 ^T (ATCC 11696)	<i>Lycopersicon esculentum</i>
<i>P. stutzeri</i>	CFBP 2443 ^T (ATCC 17588)	Spinal fluid
<i>P. syringae</i> pv. <i>atrophicus</i>	CFBP 2213 ^t	<i>Triticum aestivum</i> , New Zealand
<i>P. syringae</i> pv. <i>glycinea</i>	CFBP 2214 ^t	<i>Glycine max</i> , New Zealand
<i>P. syringae</i> pv. <i>syringae</i>	CFBP 1392 ^T	<i>Syringa vulgaris</i> , United Kingdom
<i>P. tolaasii</i>	CFBP 2152	<i>Agaricus bisporus</i> , France
<i>P. viridiflava</i>	CFBP 2107 ^T (ATCC 1323)	<i>Phaseolus</i> sp., Switzerland

^a T, type strain of the species; t, type strain of the pathovar.

roots of flax (*Linum usitatissimum* L., cv. opaline) or tomato (*Lycopersicon esculentum* Mill., cv. H63-5) cultivated in the above soil for 2-month periods alternating with 1-month periods when the soil was uncultivated. Plants were grown under greenhouse conditions in large (90-liter) containers and harvested during the fifth crop after 4 weeks of growth. Bacterial isolation was performed after the fifth crop was harvested. Four types of samples were sampled: uncultivated soil, rhizosphere, rhizoplane, and root tissue. Ten independent samples were collected for each of the four types. Each soil sample consisted of 1 g of soil, and each plant sample consisted of one root system (rhizosphere and rhizoplane) or four root systems (root tissue). Soil suspensions were prepared by vigorously shaking 1 g of soil in 9 ml of 0.1 M MgSO₄ · 7H₂O with glass beads (0.11 mm) for 60 s. Rhizosphere suspensions resulted from gently washing root systems with sterile 0.1 M MgSO₄ · 7H₂O. Rhizoplane suspensions were obtained by vigorously shaking the washed root systems for 60 s in 9 ml of 0.1 M MgSO₄ · 7H₂O. Root tissue suspensions were achieved as follows: washed root systems were surface sterilized in 0.5% (wt/vol) Ca(ClO)₂ for 10 min, rinsed four times in 0.1 M MgSO₄ · 7H₂O, and then macerated with a sterile mortar and pestle in 9 ml of 0.1 M MgSO₄ · 7H₂O. The absence of fluorescent pseudomonads on root systems was checked before maceration by plating, on King's medium B (17), suspensions obtained from root systems shaken vigorously in 9 ml of 0.1 M MgSO₄ · 7H₂O. Suspensions from the four sample types were dilution plated on modified KB (12) and incubated for 48 h at 25°C. To compare characteristics of bacterial isolates showing a similar level of dominance within each sample type, bacterial isolations from each sample type were always performed with samples diluted to the same level, i.e., 100-fold dilution (dry weight per volume) of soil or plant samples for uncultivated soil, rhizoplane, and root tissue samples and 1,000-fold dilution (dry weight per volume) of soil for rhizosphere samples. A total of 176 bacterial colonies were collected: 27 from the uncultivated soil, 26 from the flax rhizosphere, 25 from the flax rhizoplane, 29 from the flax root tissue, and 23 from each of the tomato rhizosphere, rhizoplane, and root tissue compartments. Isolates were subjected to single-colony isolation and cryopreserved at -80°C in 50% glycerol.

Biochemical and physiological tests. The presence of oxidase, fluorescent pigment production, the presence of gelatinase, arginine dihydrolase, and levansucrase, reduction of nitrate, and hypersensitive reaction on tobacco leaves were tested by the method of Lelliott et al. (23). Pectinolytic activity was assessed by the method of Prunier and Kaiser (35). Utilization of trehalose, L-(+)-tartarate, and L-tryptophan was tested by using basal medium [(NH₄)₂HPO₄, 1 g; KCl, 0.2 g; MgSO₄ · 7H₂O, 0.2 g; agar, 3 g; bromothymol blue, 0.08g; distilled water, 1,000 ml [pH 7.2]] plus each substrate at 1% (wt/vol). All these tests were applied to

differentiate the species and biovars by the methods of Stanier et al. (44) and Palleroni (32). Isolates which were categorized as belonging to neither *Pseudomonas fluorescens* nor *P. putida* were classified as belonging to an intermediate type (9).

The ability of the bacterial strains to assimilate 49 carbohydrates, 49 organic acids, and 49 amino acids was evaluated with API-50-CH, API-50-AO and API-50-AA strips (API Systems, France). The tests were performed according to the recommendations of API-bioMérieux, La Balme les Grottes, Montalieu Vercieu, France.

The similarity matrix between bacterial isolates was calculated by using the Jacquard coefficient (43) on the data from 147 substrate utilization tests and 3 enzyme activities (levansucrase, gelatinase, and nitrate reduction). Depending on their level of similarity, strains were grouped into branches (65.5%) and into clusters (76.7 or 87.8%). Clusters were made of at least four strains. Cluster analysis was performed by applying the unweighted pair group method with averages (43). Characterization of the substrates and enzymes, enabling discrimination between clusters, was achieved with the algorithm proposed by Descamps and Véron (8). This algorithm allowed the calculation of a diagnostic ability coefficient called the INF coefficient.

REP-PCR fingerprinting. Isolates were genotypically characterized by a DNA fingerprinting method based on PCR with repetitive extragenic palindromic (REP) sequences as primers (47). Bacterial cells were grown for 21 h at 25°C in liquid Luria-Bertoni medium (30) and washed with sterile ultrapure water (Millipore-Q reagent water). The cells were pelleted by centrifugation and suspended in sterile water. The cell suspensions were adjusted to an optical density at 600 nm of 2 by dilution in distilled water. Then 100 µl of 10 mM Tris-HCl (pH 8.3) and 13 µl of proteinase K (1 mg/ml in H₂O) (Sigma Chimie, St. Quentin Fallavier, France) were added to 100 µl of cell suspension, and the mixture was incubated overnight at 55°C. Proteinase K was inactivated by incubating the cell suspension for 10 min at 100°C. The REP1R-I and REP2-I oligonucleotides described by Versalovic et al. (47) were used as primers for PCR amplification. The oligonucleotides were synthesized by Eurogentec, Seraing, Belgium. The PCR was carried out with 5-µl aliquots of cell suspension under PCR conditions described previously (7), except that 1 U of *Taq* polymerase (Appligene, Strasbourg, France) was used in a 25-µl reaction volume. PCR amplifications were carried out in a TB1 TRIO-thermoblock (Biotetra, Göttingen, Germany) thermal cycler. PCR products were analyzed by horizontal electrophoresis with a 1.5% agarose gel (SeaKem; FMC, Rockland, Maine) in TAE buffer (40 mM Tris, 4 mM sodium acetate, 1 mM EDTA [pH 7.9]). Electrophoresis was done at 100 V for 5 h with 20- by 25-cm gels (Bethesda Research Laboratories model H4

FIG. 1. Dendrogram of phenotypic similarities obtained for reference strains and for isolates from uncultivated soil, from flax, and from tomato. Reference strains are indicated as follows: 1, *P. fluorescens* bv. V CFBP 2130; 2, *P. fluorescens* bv. III-1 CFBP 2127; 3, *P. fluorescens* bv. III-1 CFBP 3149; 4, *P. tolaasii* CFBP 2152; 5, *P. fluorescens* bv. V CFBP 3151; 6, *P. marginalis* CFBP 3153; 7, *P. fluorescens* bv. I-1 CFBP 2102; 8, *P. fluorescens* bv. I-1 CFBP 2123; 9, *P. fluorescens* bv. II-1 CFBP 2125; 10, *P. chlororaphis* CFBP 2132; 11, *P. aureofaciens* CFBP 2133; 12, *P. aureofaciens* CFBP 3155; 13, *P. putida* bv. B CFBP 3140-1; 14, *P. putida* bv. B CFBP 17484; 15, *P. putida* bv. A CFBP 3142-1; 16, *P. putida* bv. A CFBP 2066; 17, *P. putida* bv. A CFBP 3143-2; 18, *P. fluorescens* bv. II-1 FPFS 638; 19, *P. fluorescens* bv. IV CFBP 3150; 20, *P. fluorescens* bv. IV-1 CFBP 2129; 21, *P. viridiflava* CFBP 2107; 22, *P. syringae* pv. *atrofaciens* CFBP 2213; 23, *P. cichorii* CFBP 2101; 24, *P. syringae* pv. *syringae* CFBP 1392; 25, *P. syringae* pv. *glycinea* CFBP 2214; 26, *P. setariae* NCPPB 1392; 27, *P. stutzeri* CFBP 2443; 28, *P. saccharophila* CFBP 2433; 29, *P. palleronii* CFBP 2445; 30, *P. solanacearum* CFBP 2047.

apparatus). Gels were stained with ethidium bromide and photographed under UV illumination with Polaroid type 665 positive/negative film. Similarities among the isolates were calculated by comparing the banding patterns observed among the isolates. Banding patterns were coded by determining the total number of unique bands observed in all of the isolates examined. For each isolate,

each band position was assigned a 1 or a 0 to indicate the presence or absence of the band, respectively. For a pair of isolates, a simple matching coefficient was calculated as the sum of the number of bands present in both isolates or bands absent from both isolates, divided by the total number of bands observed in all the isolates examined. Similarity among the isolates was determined by con-

TABLE 2. Origin and taxonomic classification of bacterial isolates belonging to the phenotypic clusters defined in Fig. 1

Cluster (no. of strains)	No. of isolates from:							Species and biovar
	Soil	Rhizosphere		Rhizoplane		Root tissue		
		Flax	Tomato	Flax	Tomato	Flax	Tomato	
1 (32)	7	0	6	0	7	0	11	<i>P. fluorescens</i> bv. II
	0	0	0	0	1	0	0	Intermediate type
2 (4)	2	0	1	0	0	0	0	Intermediate type
	1	0	0	0	0	0	0	<i>P. putida</i> bv. A
3 (7)	2	3	0	0	1	0	0	<i>P. putida</i> bv. A
	0	0	0	0	1	0	0	Intermediate type
4 (10)	2	1	2	2	0	1	0	Intermediate type
	0	0	0	0	0	0	1	<i>P. putida</i> bv. A
	0	0	0	0	1	0	0	<i>P. fluorescens</i> bv. V
5 (10)	0	5	0	4	0	1	0	<i>P. putida</i> bv. A
6 (22)	0	1	6	4	2	7	2	<i>P. putida</i> bv. A
7 (5)	0	0	0	0	0	5	0	<i>P. putida</i> bv. A
8 (7)	4	0	0	1	2	0	0	<i>P. putida</i> bv. A
9 (15)	0	1	1	6	4	0	3	<i>P. fluorescens</i> bv. III
10 (10)	0	0	4	0	2	0	4	<i>P. fluorescens</i> bv. III
11 (5)	0	1	1	1	1	0	0	<i>P. fluorescens</i> bv. III
	0	1	0	0	0	0	0	<i>P. fluorescens</i> bv. II
12 (4)	0	1	0	0	0	3	0	<i>P. fluorescens</i> bv. III

structuring a dendrogram from REP-PCR data with the NTSYS-pc analysis package (version 1.8; Exeter Software, Setauket, N.Y.).

RESULTS

Comparison of phenotypic characteristics of strains belonging to different species and biovars. The 176 isolates from the uncultivated soil, the rhizosphere, the rhizoplane, and the root tissue of flax and tomato and the 30 reference strains listed in Table 1 were characterized for their ability to assimilate different substrates, their ability to reduce nitrates, and their production of gelatinase and levansucrase. Numerical analysis of these data allowed the comparison among the strains tested (Fig. 1). The level of similarity among the strains ranged between 17.3 and 95.3%. According to their level of similarity (65.5%), 94.2% of the strains tested were distributed among three branches (A, B, and C). Branch A included 36 soil and root-associated isolates. Most of them were characterized as belonging to *P. fluorescens* bv. II (88.9%). Branch B included 87 soil and root-associated isolates. Most of them (81.6%) belonged to *P. putida* bv. A. Branch C included 53 soil and

root-associated isolates. Most of them (84.9%) belonged to *P. fluorescens* bv. III. Reference strains of fluorescent *Pseudomonas* oxidase-positive species were displayed within each of the branches. Branch A comprised reference strains of *P. fluorescens* bv. I, II, III, and V, *P. tolaasii*, *P. aureofaciens*, *P. chlororaphis*, and *P. marginalis*. The last three of these species were previously called *P. fluorescens* bv. E, bv. D, and bv. B, respectively (44). Branch B included reference strains of *P. putida* bv. A and B. Branch C comprised reference strains of *P. fluorescens* bv. II and IV. Some reference strains were not aggregated in any of the three branches. These strains belonged to (i) nonfluorescent species (*P. palleronii*, *P. setariae*, and *P. solanacearum*, recently designated *Burkholderia solanacearum* [45]) and (ii) fluorescent oxidase-negative species (*P. syringae* and *P. viridiflava*). Some species are phytopathogenic (*P. cichorii*, *P. setariae*, *P. solanacearum*, *P. syringae*, and *P. viridiflava*), and some are not (*P. saccharophila* and *P. stutzeri*).

Comparison of phenotypic and taxonomic characteristics of isolates from soil, rhizosphere, rhizoplane, and root tissue. Within the three branches, strains showing a similarity of at

TABLE 3. Discrimination between isolates belonging to different clusters on the basis of substrate utilization and enzyme activities^a

Substrate or enzyme	INF coefficient ^b	% of strains able to utilize substrate and to produce enzyme in cluster ^c :											
		1	2	3	4	5	6	7	8	9	10	11	12
L-Arabinose	0.841	0	100	100	100	100	0	0	0	100	100	100	100
Gelatinase	0.829	96	100	100	100	100	0	30	0	0	0	0	0
Denitrification	0.797	100	100	100	90	100	28	0	0	0	4	0	0
Butylamine	0.788	100	100	0	0	0	14	100	100	100	100	100	100
L-Citrulline	0.736	3	100	0	0	0	100	70	100	100	81	80	0
Saccharose	0.721	100	100	100	100	100	100	100	100	0	4	0	100
N-Caproate	0.607	100	100	100	100	100	14	0	100	40	95	100	14
Isovalerate	0.584	100	20	0	20	0	0	0	25	20	95	100	0
Propionate	0.475	96	60	100	10	0	14	0	75	10	90	40	42
N-Valerate	0.470	100	46	75	50	0	0	0	25	0	86	40	0
D-Xylose	0.304	0	0	0	90	0	0	0	0	0	0	0	0
L-Aspartate	0.250	68	46	75	50	20	0	50	100	60	72	20	28

^a Tests showing the highest INF coefficient are presented.

^b Diagnostic ability coefficient.

^c Clusters defined in Fig. 1.

TABLE 4. Discrimination between bacteria isolated from different compartments on the basis of substrate utilization and enzyme activities^a

Substrate or enzyme	INF coefficient ^b	% of strains able to utilize substrate and to produce enzyme in:						
		Soil	Flax			Tomato		
			Rhizosphere	Rhizoplane	Root tissue	Rhizosphere	Rhizoplane	Root tissue
Levulinate	0.194	88	8	16	37	47	39	52
2-Cetoglutarate	0.188	60	24	32	93	65	73	86
D-Tryptophan	0.182	28	8	4	58	4	0	4
L-Arabinose	0.181	28	80	92	93	56	47	52
DL-Glycerate	0.155	100	88	100	79	78	60	47
Ethanolamine	0.154	60	16	48	44	78	78	82
Citraconate	0.142	64	84	76	100	69	43	43
Gelatinase	0.134	24	28	56	34	60	78	78
L-Aspartate	0.104	56	48	68	65	65	17	78
2-Aminobenzoate	0.103	60	24	24	72	69	39	52
Trigonelline	0.102	44	52	60	48	43	4	34
Sarcosine	0.074	76	72	100	89	91	65	91

^a Tests showing the highest INF coefficient are presented.^b Diagnostic ability coefficient.

least 87.8% were clustered. Of the 176 strains isolated from uncultivated soil, rhizosphere, rhizoplane, and root tissue, 131 were grouped in 12 clusters. The origin and taxonomic classification of strains belonging to each cluster are given in Table 2. Of the 27 soil isolates, 18 were present in 5 of the 12 clusters (clusters 1 through 4 and 8); they belonged to *P. fluorescens* bv. II (seven isolates), *P. putida* bv. A (six isolates), and the intermediate type (four isolates). Of the 97 isolates from the rhizosphere and rhizoplane, 75 were distributed in 11 of 12 clusters (clusters 1 through 6 and 8 through 12). Rhizosphere and rhizoplane isolates of the same plant species were associated except for three clusters (clusters 2, 8, and 12) and were sometimes associated with isolates from root tissue (clusters 1, 4, 5, 6, 9, 10, and 12) and more rarely associated with isolates from soil (clusters 1 through 4 and 8). Of the 52 root tissue isolates, 38 were distributed in five clusters (clusters 1, 4, 6, 9, and 10). Except for one isolate in cluster 4, isolates from flax root tissue were never associated with soil isolates. Isolates from tomato root tissue and from soil were associated only in cluster 1. Of the 150 tests used, 85 allowed differentiation of the isolates belonging to the 12 clusters. The most discriminant tests are shown Table 3. These substrates allowed differentiation of most clusters when compared one to one (0%/100%). Six pairs of clusters could not be fully discriminated. For these pairs, the best discriminative substrates were as follows: clusters 3 and 4,

D-xylose (0%/90%), propionate (100%/10%), ethanolamine (0%/90%), L-serine (25%/100%), and D-tryptophan (75%/0%); clusters 4 and 5, D-xylose (90%/0%), ribose (100%/20%), L-lysine (70%/0%), ethanolamine (90%/20%), and L-ornithine (60%/0%); clusters 6 and 7, trehalose (14%/100%), butylamine (14%/100%), mesotartrate (71%/0%), aconitate, (100%/40%), and DL-kynurenine (57%/0%); clusters 9 and 10, *n*-valerate (0%/31%), propionate (10%/90%), isovalerate (0%/86%), L-tartrate (0%/59%), and butyrate (20%/86%); clusters 10 and 11, mesaconate (18%/100%), DL-kynurenine (70%/27%), L-tryptophan (13%/80%), glycolate (36%/100%), and D-tryptophan (13%/80%); clusters 10 and 12, saccharose (4%/100%), isovalerate (95%/0%), mesotartrate (95%/0%), *n*-valerate (86%/0%), and itaconate (81%/100%).

Since isolates from each compartment were distributed in several clusters, a further analysis was performed to determine the substrates and enzymes allowing differentiation of each compartment (Table 4). The different compartments could not be fully discriminated. The values of the INF coefficient were smaller than those displayed in Table 3. These results indicated that the differentiation between compartments by each phenotypic test was less efficient than that between clusters.

TABLE 5. Origin and taxonomic classification of isolates belonging to the phenotypic clusters obtained from the comparison of flax and tomato isolates

Cluster (no. of strains)	No of isolates from:		Species and biovar
	Flax	Tomato	
1 (15)	7	8	<i>P. fluorescens</i> bv. III
2 (4)	4	0	<i>P. fluorescens</i> bv. III
3 (10)	0	10	<i>P. fluorescens</i> bv. III
4 (5)	2	2	<i>P. fluorescens</i> bv. III
	1	0	<i>P. fluorescens</i> bv. II
5 (10)	10	0	<i>P. putida</i> bv. A
6 (8)	6	2	<i>P. putida</i> bv. A
7 (14)	6	8	<i>P. putida</i> bv. A
8 (5)	5	0	<i>P. putida</i> bv. A
9 (25)	0	25	<i>P. fluorescens</i> bv. II

TABLE 6. Discrimination between isolates belonging to clusters including only flax isolates (clusters 2, 5, and 8) or including only tomato isolates (clusters 3 and 9) on the basis of substrate utilization

Substrate	INF coefficient ^a	% of strains able to utilize substrate in clusters ^b :	
		2, 5, and 8	3 and 9
Inositol	0.560	21	100
Ribose	0.560	21	100
Saccharose	0.560	21	100
Trehalose	0.560	21	100
Mesotartrate	0.547	78	0
Tryptamine	0.490	73	0
Erytritol	0.468	0	71
<i>m</i> -Hydroxybenzoate	0.468	0	71
L-Citrulline	0.463	73	2
5-Cetogluconate	0.380	5	71

^a Diagnostic ability coefficient.^b Clusters obtained from the comparison of flax and tomato isolates.

FIG. 2. REP fingerprints of 54 isolates of fluorescent pseudomonads belonging to 12 phenotypic clusters. M, molecular weight marker. The sizes (in kilobases) of the molecular size marker bands are indicated on the left of the figure.

Comparison of phenotypic and taxonomic characteristics of isolates from flax and tomato. To specifically compare isolates from flax and tomato, a further numerical analysis of substrate utilization and enzyme production by the 80 flax isolates and 69 tomato isolates was performed. This analysis allowed us to draw a dendrogram (results not shown), and strains showing a level of similarity of at least 76.7% were clustered. Of the 149 strains tested, 96 were grouped in nine clusters. The origin and taxonomic classification of isolates belonging to each cluster are shown Table 5. Of the flax isolates, 51.25% were clustered. Flax isolates were distributed in seven of the nine clusters (clusters 1, 2, and 4 through 8). They belonged to *P. fluorescens* bv. II (1 isolate) and bv. III (13 isolates) and to *P. putida* bv. A (27 isolates). Of the tomato isolates, 79.7% were clustered. Tomato isolates were distributed in six clusters (clusters 1, 3, 4, 6, 7, and 9). They belonged to *P. fluorescens* bv. II (25 isolates) and bv. III (20 isolates) and *P. putida* bv. A (10 isolates). Some clusters included only flax isolates (clusters 2, 5, and 8) or tomato isolates (clusters 3 and 9). Ten substrates allowed us to discriminate between strains belonging to these two types of clusters (Table 6).

Genotypic characterization. To assess the validity of the phenotypic groupings, a subsample of isolates was further characterized genotypically by REP-PCR fingerprinting. Fifty-four isolates were chosen to represent at least 25% of each of the 12 phenotypic clusters. Each strain produced a multiple DNA band pattern. REP fingerprints are shown in Fig. 2. With the exception of isolates belonging to clusters 8 and 12, which were not grouped to any of the three branches, the groupings obtained by comparison of REP fingerprints correlated well with those resulting from phenotypic analysis (Fig. 3). Within phenotypic clusters, most REP patterns were very similar, sharing most of their bands, but some others were quite distinct. Isolates belonging to clusters 5, 6, and 7 and representing most of the isolates assigned to *P. putida* bv. A showed obvious similarities in their REP patterns and so appeared genetically homogeneous.

DISCUSSION

Populations of fluorescent pseudomonads isolated either from uncultivated soil or from host plants showed different

phenotypic and genotypic characteristics. These differences indicated that there was significant diversity within the studied populations of fluorescent pseudomonads at both the species and the intraspecies levels. Differences between fluorescent pseudomonads from soil and roots were recorded among populations probably close to an equilibrium state as an expected result from the succession of crops of each plant species. Furthermore, thanks to our sampling strategy (10 independent samples chosen randomly per sample type, i.e., soil, rhizosphere, rhizoplane, and root tissue) and isolation procedure (all bacteria isolated from the same final dilution within each compartment), we may assume that microbial diversity was assessed in a reliable way.

Bacterial isolates were clustered on the basis of their ability to utilize different organic substrates and on the basis of their enzymatic activities. The analysis of the distribution of these isolates in different clusters as a function of their origin provided evidence that (i) the plant has a selective influence on populations of fluorescent pseudomonads, (ii) the intensity of this selection varies with the host plant, and (iii) the selection is partly plant specific.

Since 88.75% of the flax isolates were either not clustered with soil isolates (50%) or not clustered at all (38.75%), it could be concluded that most flax isolates were different from soil isolates. Differences between flax isolates and soil isolates were increasingly obvious as the isolates were more intimately associated with the host plant. Indeed, root tissue isolates and soil isolates were not aggregated. Bacterial selection achieved by tomato plants was less strongly expressed than that by flax plants. Only 50.75% of the tomato isolates were either not clustered with soil isolates (43.5%) or not clustered at all (7.25%), and root tissue and soil isolates were aggregated in cluster 1. For both plant species, isolates from the rhizosphere and rhizoplane showed similar characteristics. According to Foster and Bowen (11), the rhizoplane consists of "the actual two-dimensional surface" and the rhizosphere consists of "the adjacent volume of soil under the influence of plant roots." The width of the rhizoplane and the structure of the rhizosphere vary with the age of the root and with the distance from the root apex (10) and presumably with the plant species according to the amount of exudate being released (26). Depend-

FIG. 3. Dendrogram of 54 isolates of fluorescent pseudomonads belonging to 12 phenotypic clusters derived from REP fingerprints.

ing on the method used for recovering bacteria from roots (18), one might expect variations in the level of discrimination between bacterial populations from the rhizosphere and the rhizoplane. The lack of difference between isolates from the rhizosphere and rhizoplane recorded in the present study could thus be related to the technique used to isolate bacteria.

Tomato isolates were clustered more often than were soil or flax isolates: 92.75, 66.7, and 61.25% clustering, respectively (Table 2). These data indicate that diversity among tomato isolates was lower than that among flax isolates. The reduction of bacterial diversity recorded with isolates obtained from tomato plants is in agreement with the results of Mavingui et al. (28). These authors ascribed the lowering of the diversity of soil *Bacillus polymyxa* in the wheat rhizosphere to the bacterial selection performed by plant roots. These rhizoplane isolates were distinguished by their ability to metabolize sorbitol. In our study, differentiation between most clusters was also pos-

sible by their reactions to a small number of substrates. On the whole, the most selective substrates belonged to the organic acids and the amino acids. Discrimination of isolates belonging to different sample types was less efficient than that of isolates belonging to different clusters. This difference could be ascribed to the fact that isolates obtained from a given sample type belonged to different clusters and then showed distant phenotypes.

Since the comparison between flax and tomato (Table 5) indicated that three clusters included only flax isolates and two clusters included only tomato isolates, our results suggested that the selection achieved by the root is at least partly plant specific. Such specificity also has been established by Glandorf et al. (14) when comparing lipopolysaccharides and cell envelope protein patterns of *Pseudomonas* isolates from the rhizosphere of potato, grass, and wheat. In our study, the differentiation between flax and tomato isolates was completed with 10

substrates. As reviewed by Parke (34), the root colonization ability of microorganisms may be related to their ability to utilize root exudates, root secretions, and other materials from plants. The amount and composition of root exudates may vary from one species to another and even from one cultivar to another within one species (29), so that differences in substrate utilization between flax and tomato isolates could be related to differences in the composition of the respective root exudates. As an example, the fact that a much higher proportion of tomato isolates than of flax isolates could assimilate inositol, ribose, saccharose, trehalose, erythritol, *m*-hydroxybenzoate, and 5-cetogluconate suggests that these substrates in tomato root exudates may be important in selecting isolates specific to tomato. This hypothesis is currently being evaluated.

Species and biovars of bacterial isolates were identified by phenotypic tests described by Stanier et al. (44), Palleroni (32), and Digat and Gardan (9). Our results showed that *P. fluorescens*, *P. putida*, and the intermediate type were well distributed among the soil, rhizosphere, and rhizoplane fractions of flax and tomato. In contrast to the results of Sands and Rovira (38), showing that *P. fluorescens* bv. V was the dominant fluorescent pseudomonad in South Australian soils and wheat rhizosphere, in our study most *P. fluorescens* isolates were in bv. III. The host plant affected the fraction of each species of isolate present in the root tissue: (i) no isolates from root tissue belonged to the intermediate type, and (ii) most isolates from flax root tissue belonged to *P. putida* bv. A whereas most isolates from tomato root tissue belonged to *P. fluorescens* bv. II. Results for substrate utilization, enzyme activities, and species characterization collectively indicated that endophyte isolates were distinct from the others. Van Peer et al. (46) previously showed that from their lipopolysaccharide patterns, cell envelope proteins, and other biochemical characteristics, isolates obtained from root tissue were different from those obtained from the root surface.

Numerical analysis of data on substrate utilization and enzyme activities showed that isolates of *P. fluorescens* were distributed into two branches (A and C) whereas isolates of *P. putida* bv. A were distributed into one branch (B). These results suggested that *P. putida* bv. A was a homogeneous group and that *P. fluorescens* was a heterogeneous group. This suggestion is in agreement with our previous work showing that reference strains of *P. putida* bv. A exhibited identical 16S rDNA genotypes (21) and with previous studies on phenotypic traits and DNA-DNA homology showing that *P. fluorescens* is taxonomically heterogeneous and so far has been subdivided into six biovars (2, 5, 33). The so-called intermediate type also was distributed in different branches, suggesting that this group is not well defined. Taxonomy of the complex group of fluorescent *Pseudomonas* spp. must be clarified with the help of molecular methods such as DNA-rRNA and DNA-DNA hybridization (48) and restriction analysis of 16S rDNA (21).

A subsample of the bacterial isolates also was genotypically characterized by a molecular method based on analysis of polymorphism in DNA. REP fingerprinting was chosen because this method involves the use of PCR and primers derived from repetitive sequences scattered throughout the genome. Hence, it provides a rapid and universal tool to assess genomic variations in prokaryotic organisms (47) and reflects the variability of the overall genome. Moreover, this method is highly discriminant, allowing the differentiation of very closely related bacterial strains within species (7). On the whole, a positive correlation was found in this study between groupings of isolates established by the genotypic and phenotypic analyses. These data indicated that the phenotypic characterization was representative of the overall genetic relatedness among the

fluorescent *Pseudomonas* isolates examined in this study. Data from multilocus enzyme electrophoresis, REP fingerprinting, and phenotypic characterization of *Rhizobium leguminosarum* strains were also correlated (25).

Our results suggest that the ability of fluorescent pseudomonads to utilize specific organic substrates may be one important bacterial trait involved in the selection of soilborne fluorescent pseudomonads achieved by the plant. When introduced into the rhizosphere, a bacterial inoculant having the ability to utilize a compound present in the root exudate should have a competitive advantage over soil bacteria without this ability (6, 22). Other bacterial traits including cell surface properties, mobility, osmotolerance, and growth rate have also been proposed to play a role in root colonization (13). The relationship between the selection by a host plant, as a result of the production of specific organic compounds, and the rhizosphere competence of isolates showing the ability to use these specific compounds has yet to be demonstrated. This relationship could be examined by comparing root colonization by the wild type and by mutants auxotrophic for utilization of exudate compounds (31, 42).

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