The Composition of Fluorescent *Pseudomonad* Populations Associated with Roots Is Influenced by Plant and Soil Type

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Populations of fluorescent pseudomonads isolated from an uncultivated soil and from the roots of two plant species were previously shown to differ (P. Lemanceau, T. Corberand, L. Gardan, X. Latour, G. Laguerre, J.-M. Boeufgras, and C. Alabouvette, *Appl. Environ. Microbiol.* 61:1004–1012, 1995). The diversities of fluorescent pseudomonads, from two uncultivated soils and from the roots of two plant species cultivated in these two soils, were compared. The phenotypic diversity of the bacterial isolates was characterized on the basis of biochemical and physiological tests and on the basis of their ability to utilize 147 different organic compounds. The genotypic diversity of the isolates was characterized on the basis of the types of 16S genes coding for rRNA (rDNA), their repetitive extragenic palindromic patterns by PCR, and plasmid profiles. Taxonomic identification of the isolates was achieved with both biochemical and physiological tests and by comparing their 16S rDNA types to those of reference and type strains of fluorescent *Pseudomonas* spp. Numerical analysis of phenotypic characteristics allowed the clustering of isolates that showed high levels of similarity. This analysis indicated that both soil type and host plant had an effect on the diversity of fluorescent pseudomonads. However, of the two factors studied, the soil was clearly the dominating one. Indeed, the populations associated with the roots of each plant species varied from one soil to the other. This variation could possibly be ascribed to the differences recorded between the phenotypically diverse populations of fluorescent pseudomonads from the two uncultivated soils. The plant selection was, at least partly, plant specific. It was not related to bacterial species and biovars or to the presence of plasmid DNA. The phenotypic clustering of isolates was well correlated with genotypic characterization by repetitive extragenic palindrome-PCR fingerprinting.

Fluorescent pseudomonads can suppress various soilborne diseases (52). Their efficacy has been related both to their antagonistic activities and to their rhizospheric competence (12, 16). Overall, biological control of soilborne diseases achieved by fluorescent pseudomonads is often inconsistent (28, 52). This inconsistency has been partially associated with inefficient root colonization by the introduced bacteria (7, 43). Knowledge about bacterial traits promoting root colonization is lacking. In order to generate hypotheses, the characteristics of fluorescent pseudomonad populations associated with the roots of two plant species (flax and tomato) have recently been compared with those of fluorescent pseudomonads from an uncultivated soil (11, 29). The results from these studies, performed with the same soil, indicated that bacterial populations are not distributed at random. Indeed, populations from the roots of each plant species and from the uncultivated soil differed in their abilities to use different organic compounds and to dissimilate nitrogen. These differences suggest that the plant selects specific populations of fluorescent pseudomonads.

Even if the plant plays a major role on the populations associated with its root system, the influence of the soil cannot be excluded. Several studies have clearly shown that the survival of different introduced strains of fluorescent pseudomonads varies in soils of different textures (2, 48, 49). However, knowledge about the effect of the soil type on the

selection achieved by the host plant toward the soilborne populations of fluorescent pseudomonads is missing.

The aim of the present study was to determine the influence of the soil on (i) the diversity of the populations of fluorescent pseudomonads associated with the roots of two plant species and (ii) the bacterial traits related to the selection exerted by the plant toward fluorescent pseudomonads. The phenotypic and genotypic diversities of fluorescent pseudomonads associated with the roots of flax and tomato cultivated in two different soils have been compared with each other and with those of fluorescent pseudomonads of the corresponding uncultivated soils.

MATERIALS AND METHODS

Soil characteristics. The main characteristics of the two soils used in the present study are listed in Table 1. The soils from Châteaurenard (France) and Dijon (France) are calcic silt-clay and clay-silt soils, respectively. The CaCO₃ content and pH of the Châteaurenard soil are much higher than those of the Dijon soil. The Châteaurenard soil has a higher microbial biomass and a higher level of activity (specific respiration) than the Dijon soil. The Châteaurenard soil is known for its natural suppressiveness towards fusarium wilts (32), whereas the Dijon soil is conducive to these soilborne diseases.

Bacterial strains. Bacterial strains were isolated from either uncultivated soils of Châteaurenard and Dijon or the roots of flax (*Linum usitatissimum* L., cv. opaline) or of tomato (*Lycopersicon esculentum* Mill., cv. H63-5) cultivated, in these same soils, for 2-month periods alternated with 1-month periods of no cultivation. Plants were grown under greenhouse conditions in large containers (90 liters) and harvested during the fifth crop after 4 weeks of growth. Bacterial isolation was done after the fifth crop. Four compartments were sampled: uncultivated soil, rhizosphere, rhizoplane, and root tissue. The procedure of isolation was performed as previously described (29). From the Châteaurenard soil, 167 bacterial colonies were collected from the four compartments: 25 from the uncultivated soil, 25 from the flax rhizosphere, 25 from the flax rhizoplane, 17 from the flax root tissue, and 25 from each of the tomato rhizosphere, rhizoplane, and root tissue compartments. From the Dijon soil, 173 bacterial colonies were

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TABLE 1. Main characteristics of the soils used in this study

Soil characteristic	Châteaurenard soil	Dijon soil
Mechanical analysis of soil (<2,000 μm %)		
Clay (<2 μm)	14	35.1
Fine loam (2–20 μm)	9.6	24.6
Coarse loam (20–50 μm)	13.5	22.4
Fine sand (50–200 μm)	12.7	12.1
Coarse sand (200–2,000 μm)	13.5	3
pH	8.05	6.91
Organic C (%)	0.87	1.22
CaCO ₃ (%)	38.9	0
Biomass of C (mg of C kg ⁻¹)	727	376
Specific respiration (mg of C kg ⁻¹ J ⁻¹)	13.4	5.8

collected from the four compartments: 25 from the uncultivated soil, 25 from the flax rhizosphere, 25 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. Additional type and reference strains belonging to different fluorescent *Pseudomonas* species were included in the present study. Most of them have been described previously (25): *P. aeruginosa* CFBP 2466; *P. chlororaphis* CFBP 2132; *P. chlororaphis* (formerly *P. aureofaciens*) CIP 103295; *P. cichorii* CFBP 2101; *P. fluorescens* bv. I CFBP 2102, bv. II CFBP 2126, bv. III CFBP 2127, bv. IV CFBP 2129, bv. V CFBP 2131, and bv. VI CFBP 2124; *P. putida* bv. A CFBP 2066; *P. putida* bv. B ATCC 17484; *P. putida* bv. C CFBP 2130; and *P. tolaasii* CFBP 2068.

Biochemical and physiological tests. The presence of oxidase, fluorescent pigment production, gelatinase, arginine dihydrolase, levan-sucrase, reduction of nitrate, and hypersensitive reactions on tobacco leaves were tested according to the methods of Lelliott et al. (26). Pectinolytic activity was assessed by the method of Prunier and Kaiser (39). Utilization of trehalose, L-tartrate, and L-tryptophan was tested in basal medium (NH₄H₂PO₄, 1 g; KCl, 0.2 g; MgSO₄ · 7H₂O, 0.2 g; agar, 3 g; bromothymol blue, 0.08 g; distilled water, 1,000 ml [pH 7.2]) plus each substrate at 1% (wt/vol). All these tests were used in order to differentiate the species and biovars according to the methods of Stanier et al. (46), Palleroni (38), Champion et al. (10), and Barrett et al. (4).

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

The similarity matrix between bacterial isolates was calculated by using the Jacquard coefficient (45) with the data from 147 substrate utilization tests and for three enzyme activities (levan-sucrase, gelatinase, and denitrification). Clusters were made of at least 5 strains. Cluster analysis was performed by applying the unweighted-pair-group method with averages (45). Characterization of the substrates and enzymes enabling discrimination between clusters was achieved with the algorithm proposed by Descamps and Veron (15). This algorithm allowed the calculation of a diagnostic ability coefficient called the INF coefficient.

REP-PCR fingerprinting. Wild-type isolates were genotypically characterized by a DNA fingerprinting method based on the PCR with repetitive extragenic palindromic (REP) sequences as primers (50). The procedure of in vitro amplification of the DNA by PCR and conditions for electrophoresis have been described previously (29). Gels were stained with ethidium bromide and photographed under UV illumination with Polaroid type 665 positive/negative film.

PCR amplification and restriction fragment length polymorphism (RFLP) analysis of 16S rDNA. The 16S rDNA genes (rDNAs) were amplified by PCR, with the primer pair fD1 and rD1 (51), and digested with 13 restriction endonucleases as previously described (25).

Plasmid profiles. The bacterial strains were grown for 24 h at 25°C in liquid King's B medium (23). The cells were pelleted by centrifugation, suspended in sterile water, and adjusted to an A_{600} of 1. In order to detect a wide range of plasmid sizes, two different methods of resolving plasmids were used: the Eckhardt method (17) as modified by Simon et al. (44) and the Kado and Liu method (22). The principle of the Eckhardt method is based on a mild lysis of cells directly in wells of agarose gels, which preserves the high-molecular-weight plasmids such as *Rhizobium meliloti* megaplasmids (about 1,000 MDa [8]). Preparation of plasmid DNA by the alkaline lysis, described by Kado and Liu (22), allows the resolution of plasmids with sizes between 2.6 and 350 MDa.

RESULTS

Comparison of phenotypic characteristics of isolates from the two uncultivated soils. The 50 isolates from the unculti-

FIG. 1. Dendrogram of phenotypic similarities obtained for isolates from uncultivated soils of Châteaurenard and Dijon.

vated soils of Châteaurenard and Dijon were characterized for their ability to assimilate different substrates and to denitrify and for their production of gelatinase and levan-sucrase. Numerical analysis of these data was done to compare the populations (Fig. 1). The level of similarity among the strains ranged from 66.8 to 100%. According to their level of similarity (80%), 96% of the strains from the Châteaurenard soil and 52% of the strains from the Dijon soil were distributed in two clusters each (Table 2). No cluster included isolates from the two soils.

Comparison of phenotypic characteristics of isolates from the uncultivated soil of Châteaurenard and from the rhizosphere, rhizoplane, and root tissues of flax and tomato cultivated in this soil. The comparison of the phenotypic characteristics of isolates, from the Dijon soil and from the rhizosphere, rhizoplane, and root tissue of flax and tomato cultivated in this soil, has been reported previously (29). Numerical analysis of substrate utilization and enzyme production by the 25 soil isolates, 67 flax isolates, and 75 tomato isolates from the Châteaurenard soil was performed in the present study. The level of similarity among the strains ranged between 68 and 100%. A dendrogram was constructed from the similarity matrix (results not shown), and strains showing a level of similarity of at least 91.5% were clustered. One hundred forty-six of the 167 strains tested were grouped in nine clusters. The

TABLE 2. Origin, 16S rDNA types, and taxonomic classification of bacterial isolates belonging to phenotypic clusters resulting from comparison of isolates from uncultivated soils of Châteaurenard and Dijon

Cluster (no. of strains)	No. of isolates from:		16S rDNA type(s)	Species and biovar ^a (no. of strains)
	Châteaurenard soil	Dijon soil		
1 (19)	19	0	C1	<i>P. putida</i> bv. A (19)
2 (6)	0	6	D1	<i>P. putida</i> bv. A (6)
3 (5)	5	0	C3/C2, C3	<i>P. fluorescens</i> bv. II (4) <i>P. fluorescens</i> bv. IV (1)
4 (7)	0	7	D2	<i>P. chlororaphis</i> (7)

^a Species and biovars were determined on the basis of biochemical tests.

TABLE 3. Origin, 16S rDNA types, and taxonomic classification of bacterial isolates belonging to phenotypic clusters resulting from comparison between isolates from uncultivated soil of Châteaurenard and isolates from rhizosphere, rhizoplane, and root tissues of flax and tomato cultivated in Châteaurenard soil

Cluster (no. of strains)	No. of isolates from:							16S rDNA type(s)	Species and biovar ^a (no. of strains)
	Flax				Tomato				
	Soil	Rhizosphere	Rhizoplane	Root tissue	Rhizosphere	Rhizoplane	Root tissue		
1 (10)	1	0	0	8	1	0	0	C3/C2, C4	<i>P. fluorescens</i> bv. II (10)
2 (17)	2	3	8	1	1	2	0	C3/C2	<i>P. fluorescens</i> bv. II (11) <i>P. fluorescens</i> bv. IV (6)
3 (5)	0	1	2	0	0	2	0	C3/C2	<i>P. fluorescens</i> bv. II (5)
4 (13)	13	0	0	0	0	0	0	C1	<i>P. putida</i> bv. A (13)
5 (5)	3	1	1	0	0	0	0	C1	<i>P. putida</i> bv. A (5)
6 (36)	0	15	12	9	0	0	0	C5	<i>P. fluorescens</i> bv. VI (36)
7 (19)	0	0	0	0	7	7	5	C5	<i>P. fluorescens</i> bv. VI (19)
8 (35)	0	0	0	0	6	9	20	C6	<i>P. fluorescens</i> bv. II (35)
9 (6)	1	0	1	0	4	0	0	C7/C8	<i>P. fluorescens</i> bv. II (6)

^a Species and biovars were determined on the basis of biochemical tests.

origins of isolates belonging to each cluster are shown in Table 3. Eighty percent of the soil isolates, 88% of the flax isolates, and 85.3% of the tomato isolates were distributed in five, six, and six clusters, respectively. Some clusters (clusters 1, 2, and 9) included isolates from uncultivated soil, flax, and tomato. Cluster 5 included both soil and flax isolates, and cluster 3 included both flax and tomato isolates. Other clusters (clusters 6, 7, and 8) were specific to the plant species: cluster 6 included only flax isolates (36 isolates), and clusters 7 and 8 included only tomato isolates (54 isolates). Flax and tomato isolates from the rhizosphere and rhizoplane were distributed in five and six clusters, respectively, whereas flax and tomato isolates from root tissues were distributed in only three and two clusters, respectively.

Comparison of phenotypic characteristics of isolates from the rhizosphere, rhizoplane, and root tissues of flax and tomato cultivated in the soils of Châteaurenard and Dijon. To assess the possible effect of the soil on the selection achieved

by the two plant species, numerical analysis of substrate utilization and enzyme production by the 67 flax isolates and the 75 tomato isolates from the Châteaurenard soil and by the 79 flax isolates and the 69 tomato isolates from the Dijon soil was performed. A dendrogram was constructed from the similarity matrix (results not shown), and strains showing a level of similarity of at least 82% were clustered. Two hundred ten of the 290 strains tested were grouped in 12 clusters. The origins of the isolates belonging to each cluster are shown in Table 4. Isolates from plants grown in the Châteaurenard soil were never associated with those from plants grown in the Dijon soil. Of the isolates from the Châteaurenard soil, two clusters (clusters 3 and 8) included flax and tomato isolates, one cluster (cluster 1) included only flax isolates (36 isolates), and two others (clusters 2 and 6) included only tomato isolates (56 isolates). Of the isolates from the Dijon soil, four clusters (clusters 4, 7, 10, and 11) included both flax and tomato isolates, one cluster (cluster 9) included only flax isolates (7 iso-

TABLE 4. Origin and 16S rDNA types of bacterial isolates belonging to phenotypic clusters resulting from comparison between isolates from rhizosphere, rhizoplane, and root tissues of flax and tomato cultivated in Châteaurenard and Dijon soils

Cluster (no. of strains)	No. of isolates from ^a :												16S rDNA type(s)	Species and biovar ^b (no. of strains)
	Châteaurenard soil						Dijon soil							
	Flax			Tomato			Flax			Tomato				
	R	Rp	RT	R	Rp	RT	R	Rp	RT	R	Rp	RT		
1 (36)	15	12	9	0	0	0	0	0	0	0	0	0	C5	<i>P. fluorescens</i> bv. VI (36)
2 (21)	0	0	0	8	8	5	0	0	0	0	0	0	C5	<i>P. fluorescens</i> bv. VI (21)
3 (34)	7	11	9	3	4	0	0	0	0	0	0	0	ND ^c	<i>P. fluorescens</i> bv. II (29)
4 (5)	0	0	0	0	0	0	2	1	0	1	1	0	ND	<i>P. fluorescens</i> bv. IV (5)
														<i>P. fluorescens</i> bv. II (2)
														<i>P. fluorescens</i> bv. IV (3)
5 (7)	0	0	0	0	0	0	0	0	0	2	1	4	D3	<i>P. fluorescens</i> bv. II (5)
														<i>P. fluorescens</i> bv. IV (2)
														<i>P. fluorescens</i> bv. IV (2)
6 (35)	0	0	0	6	9	20	0	0	0	0	0	0	C6	<i>P. fluorescens</i> bv. II (35)
7 (15)	0	0	0	0	0	0	1	6	0	1	4	3	ND	<i>P. fluorescens</i> bv. IV (15)
8 (9)	0	1	0	5	3	0	0	0	0	0	0	0	ND	<i>P. fluorescens</i> bv. II (9)
9 (7)	0	0	0	0	0	0	4	3	0	0	0	0	D1, D4	<i>P. putida</i> bv. A (7)
10 (7)	0	0	0	0	0	0	1	2	2	1	1	0	ND	<i>P. putida</i> bv. A (7)
11 (9)	0	0	0	0	0	0	0	1	2	3	1	2	ND	<i>P. putida</i> bv. A (9)
12 (25)	0	0	0	0	0	0	0	0	0	6	8	11	D5	<i>P. chlororaphis</i> (25)

^a R, rhizosphere; Rp, rhizoplane; RT, root tissue.

^b Species and biovars were determined on the basis of biochemical tests.

^c ND, not determined.

TABLE 5. Discrimination between bacterial isolates belonging to phenotypic clusters resulting from comparison between isolates from rhizosphere, rhizoplane, and root tissues of flax and tomato plants cultivated in Châteaurenard and Dijon soils

Substrate, process, or enzyme	% of isolates able to utilize substrate and to produce enzyme in:						INF coefficient ^a
	Châteaurenard soil			Dijon soil			
	Flax (cluster 1)	Tomato		Flax (cluster 9)	Tomato		
		Cluster 2	Cluster 6		Cluster 5	Cluster 12	
Inositol	0	0	100	0	100	100	0.919
Denitrification	0	0	100	0	100	100	0.919
Trehalose	0	100	8	0	100	100	0.865
Saccharose	0	9	100	0	100	100	0.859
Levan-sucrase	0	0	100	0	100	96	0.809
D-Xylose	0	95	100	0	85	0	0.794
D-Tartrate	0	90	100	14	0	0	0.699
Tryptamine	0	0	0	100	0	0	0.584
L-Citrulline	0	0	0	100	0	4	0.569

^a Diagnostic ability coefficient.

lates), and two others (clusters 5 and 12) included only tomato isolates (32 isolates).

Clusters including only flax isolates or only tomato isolates could be discriminated on the basis of their ability to utilize different substrates and their differences in enzymatic activities (Table 5). Substrates and enzymes allowing discrimination of the two plant species varied from one soil to the other. Some substrates (inositol, trehalose, and saccharose) were utilized and some enzymatic activities were expressed by all tomato isolates (denitrification) or by most of tomato isolates (levan-sucrase) from Dijon soil but by only a part of the tomato isolates from Châteaurenard soil. In contrast, D-xylose was used by most of the tomato isolates from Châteaurenard soil but by only some tomato isolates from Dijon soil. Lastly, D-tartrate was used by most of the tomato isolates from Châteaurenard soil but by none of the tomato isolates from Dijon soil; tryptamine and L-citrulline were used by all flax isolates from Dijon soil but by none of the flax isolates from Châteaurenard soil.

REP-PCR DNA fingerprinting. Three subsamples of isolates were chosen to represent 25% of the isolates included in clusters resulting from the three phenotypic analyses. The first subsample, chosen from the clusters resulting from the comparison of the two uncultivated soils, included 13 isolates. The second subsample, chosen from the clusters resulting from the comparison of the uncultivated soil of Châteaurenard and the flax and tomato plants cultivated in this soil, included 38 isolates. The third subsample, chosen from the clusters resulting from the comparison of isolates from plants cultivated in both soils but grouped as only either flax or tomato isolates, included 37 isolates. Each of the isolates sampled produced a multiple DNA band pattern. Within the phenotypic clusters, most REP-PCR patterns were very similar, sharing most of their bands. Different REP-PCR patterns were clustered in REP groups: 6 among 4 clusters, 10 among 9 clusters, and 8 among 6 clusters in the first, second, and third subsamples, respectively. Phenotypic clusters and REP-PCR groups were more highly related among the isolates from the Châteaurenard soil than among those from the Dijon soil. Except for two clusters, each Châteaurenard cluster included isolates sharing similar REP-PCR patterns whereas the Dijon clusters included isolates sharing one or two different REP-PCR patterns. As an example, Fig. 2 shows REP-PCR patterns of the 38 isolates from the second subsample, clustered in nine REP-PCR groups. Cluster 4, including only soil isolates, cluster 6, including only flax isolates, and clusters 7 and 8, including only

tomato isolates, all belonged to different REP-PCR groups, d, f, g, and h, respectively. In contrast, cluster 1 included isolates belonging to two different REP-PCR groups (a and b), and clusters 2 and 3 belonged to the same REP-PCR group (c).

Taxonomic characterization. Classification of the isolates at the species and biovar levels was achieved by using both traditional biochemical and physiological tests (4, 10, 38, 46) and by PCR-RFLP analysis of 16S rDNA as previously described (25).

Results from the phenotypic characterization indicated that *P. putida* was the most frequently isolated bacterial species in both uncultivated soils: 76 and 48% of the isolates of Châteaurenard and Dijon, respectively, belonged to this species. The two soils could be distinguished at the species level by the absence (in the Châteaurenard soil) or presence (in the Dijon soil [28%]) of isolates belonging to *P. chlororaphis*. The proportion of *P. fluorescens* within plant isolates was higher than within soil isolates (Table 2). In the Châteaurenard soil, 97 and 100% of the flax and tomato isolates, respectively, and only 24% of the soil isolates belonged to *P. fluorescens* (Table 3).

Twenty-nine isolates representing the different REP-PCR groups were analyzed by PCR-RFLP of 16S rDNA with the 13

FIG. 2. REP fingerprints of 38 isolates of fluorescent pseudomonads belonging to nine phenotypic clusters, resulting from comparison between isolates from uncultivated soil of Châteaurenard and isolates associated with flax and tomato plants cultivated in this soil and clustered in nine REP-PCR groups. M, molecular size markers, with the sizes of the bands indicated on the left.

restriction endonucleases used in a previous work (25). Depending on the restriction endonucleases and the strains, three to eight restriction fragments were recorded. The map locations of restriction sites in the 16S rRNA gene were inferred from the known rDNA sequences of *P. aeruginosa*, *P. mendocina*, and *P. putida* and the previously published data (25). Among the *Pseudomonas* strains tested, the combined data from the 13 endonucleases gave 13 composite 16S rDNA types. For six strains, the purities of which were carefully checked, the sum of the restriction fragments from *HinfI* restriction patterns was higher than that of the uncut PCR products. A part of these fragments corresponded to a restriction pattern shared by several other 16S rDNA types. The additional fragments constituted distinct restriction patterns which defined 16S rDNA types C2 and C8. The two restriction patterns differed by only one restriction site. This result can be explained by intragenomic variation between the copies of the 16S rRNA gene in individual strains, as previously reported for other bacterial species (31, 35). In our study, such strains were identified by the double 16S rDNA types (e.g., C3/C2 and C7/C8). One hundred percent and 37.5% of the Châteaurenard isolates from clusters including only flax isolates and only tomato isolates, respectively, were identified as *P. fluorescens* bv. VI by biochemical tests and belonged to the same 16S rDNA type (C5) (Table 3).

In order to evaluate the genetic relationships between the 16S rDNA types of the wild-type isolates and the reference and type strains of fluorescent *Pseudomonas* spp., Dollo parsimony analysis (47) of the data matrix was done (data not shown). The data for the reference and type strains have been published previously (25). Among the 105 restriction sites analyzed, which represent 420 bp, 43 were polymorphic within the sample of *Pseudomonas* spp. strains. One hundred equally parsimonious trees were obtained from a heuristic search. These trees showed few differences in topology; one of these trees is presented in Fig. 3. Twenty-four 16S rDNA types were delineated (Fig. 3). The 16S rDNA type D5 of wild isolates identified phenotypically as *P. chlororaphis* was identical to that of the *P. chlororaphis* type strain (Chlo1), and the 16S rDNA type D2 of the other isolates identified as *P. chlororaphis* differed by only one restriction site from the type strain. Isolates identified as *P. fluorescens* bv. IV (C2, C3, and D3) differed by only one to three restriction sites from the corresponding reference strain (FIV), but the D3 type of *P. fluorescens* bv. IV was also closely related to the *P. chlororaphis* type strain and reference strain (Chlo2, formerly the *P. aureofaciens* type strain). All the isolates tested identified as *P. fluorescens* bv. VI shared 16S rDNA type C5, and the most closely related 16S rDNA type among the reference strains was indeed that of *P. fluorescens* bv. VI (FVI), with only two differences in the restriction sites. However, the 16S rDNA types of isolates identified as *P. fluorescens* bv. II (C2 to C8 and D3) were not closely related to that of the corresponding reference strain (FII) (5 to 10 differences in restriction sites). Likewise, 16S rDNA types of isolates identified as *P. putida* bv. A (C1, D1, and D4) differed from that of the corresponding reference strain (PuA) by five or six restriction sites but were closely related to the reference strains of *P. fluorescens* bv. VI and *P. putida* bv. B (PuB) (one or two differences in restriction sites).

Plasmid content. Plasmid analysis was performed on the subsamples used for the REP-PCR DNA fingerprinting. Among the two methods tested (17, 22, 44), the best visualization of plasmids among reference strains was achieved by the one described by Kado and Liu (22). The frequency of soil and root-associated fluorescent pseudomonad isolates harboring plasmids was extremely low: only two isolates from the uncultivated

FIG. 3. Tree constructed by parsimony analysis showing relationships between 16S rDNA types: FI, *P. fluorescens* bv. I; FIII, *P. fluorescens* bv. III; FII, *P. fluorescens* bv. II; FV, *P. fluorescens* bv. V; Tol, *P. tolaasii*; Chlo1, *P. chlororaphis*; Chlo2, *P. chlororaphis* (formerly *P. aureofaciens*); FIV, *P. fluorescens* bv. IV; Cic, *P. cichorii*; PuA, *P. putida* bv. A; FVI, *P. fluorescens* bv. VI; PuB, *P. putida* bv. B; PuC, *P. putida* bv. C; Aer, *P. aeruginosa*; C1 to C8 and D1 to D5, 16S rDNA types from wild-type isolates. The horizontal branches are drawn proportionally to the numbers of restriction sites changes. The tree length was 90 steps. The taxonomic identifications of wild-type isolates on the basis of biochemical tests are indicated within parentheses.

tivated soils (one from Châteaurenard soil and one from Dijon soil), four flax isolates (three from Châteaurenard soil and one from Dijon soil), and none of the tomato isolates. The number and the estimated molecular size of plasmids harbored by these strains ranged from 1 or 2 and from 22 to 210 MDa, respectively (data not shown).

DISCUSSION

The phenotypic and genotypic diversities of populations of fluorescent pseudomonads from two uncultivated soils and from the roots of two plant species cultivated in these two soils were compared.

The genotypic characterization of the isolates based on REP-PCR DNA fingerprinting was more discriminant than PCR-RFLP analysis of 16S rDNA types. Several REP-PCR groups could be identified within each 16S rDNA type, but different 16S rDNA types never included similar REP-PCR patterns (results not shown). Similar levels of discrimination were achieved by REP-PCR DNA fingerprinting and by numerical analysis of phenotypic data. As previously reported (29), both characterizations were well correlated. In contrast, neither the specific trophic traits of the isolates nor their origins could be related to the presence of plasmid DNA. In agreement with previous studies (6, 9, 36), the frequency of fluorescent pseudomonad isolates harboring plasmids was extremely low.

Identification of the bacterial isolates was done by biochemical and physiological tests and by RFLP analysis of 16S rDNA. Recently, Laguerre et al. (25) have shown that PCR amplification of 16S rDNA followed by restriction analysis could be an appropriate method to differentiate species within the genus *Pseudomonas*. The phenotypic identification of *P. chlororaphis* conformed well with the results of PCR-RFLP analysis of 16S rDNA. A similarly close relationship was also established among isolates of the pathogenic species *P. fuscovaginae* (21). In contrast, as already reported by Laguerre et al. (25), intraspecies and even intrabiovar variations were observed in *P. fluorescens* and *P. putida*. Overall, our results show the lack of relationship between the species and biovar identifications of *P. fluorescens* and *P. putida* isolates on the basis of biochemical traits and 16S rDNA types. Discrimination of isolates belonging to *P. fluorescens* bv. II and IV was difficult. The 16S rDNA types of these isolates were closely related. In the same way, according to Palleroni (38), the differentiation between the two biovars was based only on their ability to utilize one organic compound (L-tartrate). So, isolates belonging to biovars II and IV were often grouped together in the same phenotypic clusters, whereas all the other clusters included isolates belonging to only one species and biovar. The taxonomies of *P. fluorescens* and *P. putida* need to be clarified by molecular methods based on DNA-DNA homologies.

Populations of fluorescent pseudomonads isolated either from uncultivated soils 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 intraspecies levels. The analysis of the distribution of the isolates in different clusters as a function of their origins provided evidence that (i) the plant has a selective influence on populations of fluorescent pseudomonads, (ii) the populations of fluorescent pseudomonads differ from one uncultivated soil to the other, and (iii) the populations associated with the roots of a plant species differ from one soil to the other.

The selection of fluorescent pseudomonads by two plant species, already shown for the Dijon soil (29), was confirmed for the Châteaurenard soil. Indeed, most plant isolates were different from soil isolates. Furthermore, since most isolates associated with one plant species differed from those associated with the other plant species, the selection achieved by the roots was, at least partly, plant specific. As previously described, the ability of fluorescent pseudomonads to utilize specific organic substrates may be one important bacterial trait in the selection of soilborne fluorescent pseudomonads achieved by the plant. The discrimination between the clusters including only flax isolates and only tomato isolates was completed with two substrates (Table 5). This selection was not related to specific species or biovars of fluorescent pseudomonads or to 16S rDNA types. As an example, isolates from flax and tomato plants cultivated in Châteaurenard soil, included in two different clusters (clusters 6 and 7), belonged to the same species, biovar, and 16S rDNA type (Table 3). The plant selection was not associated with the presence of plasmid DNA. For both plant species, isolates from the rhizosphere and the rhizoplane were included in the same clusters and then showed similar characteristics. Isolates from root tissues were distributed in fewer clusters than were the rhizosphere and rhizoplane isolates. These results indicated that the diversity among endophytic isolates was less than that among rhizospheric and rhizoplane isolates. This finding suggests that the selection achieved by the plant is stronger for the endophytic isolates than for the rhizosphere and rhizoplane isolates.

Even in the absence of any selective pressure by the plants

during a period of 1 year, the two soils studied harbored different populations of fluorescent pseudomonads. Indeed, no cluster included isolates from both the uncultivated soils of Châteaurenard and Dijon (Table 2). This result suggests that the soil characteristics affect the populations of fluorescent pseudomonads. Châteaurenard and Dijon soils differ in their natural suppressiveness towards fusarium wilts: Châteaurenard soil is suppressive whereas Dijon soil is conducive. Since the suppressiveness towards fusarium wilts was partly associated with the fluorescent pseudomonads (24, 42), one may expect the populations from the suppressive soil and from the conducive soil to differ. However, the relationship between the differences in the soil characteristics and the differences in the bacterial populations between the 2 soils are difficult to establish. To our knowledge, the few reports on the comparison of populations of fluorescent pseudomonads between two soils resulted from studies with cultivated soils (30, 41). Different studies were undertaken to compare the survival of introduced strains of fluorescent pseudomonads in soils with different textures (19, 48, 49). Fine textures or soils amended with clay favor the survival of the introduced strains. Höper et al. (20) have also shown that an increase of the soil pH and amendment of a soil (pH 7) with clay (montmorillonite or illite) induces a significant increase of the density of the endogenous populations of fluorescent pseudomonads. The two soils used in the present study differ greatly both in their pH, 8.05 and 6.91 for the Châteaurenard and the Dijon soils, respectively, and in their textures. This difference may affect the populations of fluorescent pseudomonads. Besides their survival, the levels of activity of fluorescent pseudomonads have also been related to soil characteristics. Suppression of fusarium wilts by siderophore-mediated iron competition has been associated with a low concentration of iron available for the pathogen (27); this low availability is related to a high pH and a high CaCO_3 content in the suppressive soils. The disease suppression related to phenazine production by *P. fluorescens* strain 2-79 has been positively correlated with the percentage of sand and pH and negatively correlated with iron and the percentages of silt, clay, and organic matter (37). The diversity within bacterial populations was higher in the Dijon soil than in the Châteaurenard soil. This difference between the two soils could be related to the stronger selective pressure which is achieved in the Châteaurenard soil than in the Dijon soil. Indeed, the intensity of the carbon and iron competition has been demonstrated to be higher in the suppressive soil than in the conducive soil. This difference was related both to the higher levels of microbial activity and reactivity and to the low concentration of available iron (1, 27).

Fluorescent pseudomonads associated with each of the two plant species, flax and tomato, differed between the two soils. Indeed, no cluster included isolates from both plant species cultivated in Châteaurenard soil and in Dijon soil. Since the populations of the two uncultivated soils were different, one may expect the populations selected by a plant species among the soil populations to also differ in the two soils. The difference between the populations selected by a plant species cultivated in the two soils could also be ascribed to variations in the amounts and compositions of the compounds exuded in relation to the soil characteristics. The texture of the two soils differed significantly. This difference would affect the mechanical impedance which has been shown to affect the root exudation (5). The availability of the root exudates may vary in the two soils showing different clay contents. Indeed, some amino acids and peptides may be adsorbed and bound on clay minerals (13, 14) and thus used to a lesser extent than when they are free in solution (18). Besides the physical properties of the

soils, the biomass and activity of the microflora, which are both higher in the Châteaurenard soil than in the Dijon soil, may modify the root exudation. Microorganisms were shown to stimulate the amount (33, 40) and to affect the composition (34) of the root exudates.

As suggested by Bachman and Kinzel (3), the soil was clearly the dominating factor, in the combination of soil and plant, responsible for the diversity of the bacterial populations associated with the roots. Further studies are required to determine if the soil characteristics interfere with the selection by the host plant as a possible result of the production of specific organic compounds. The possible effect of the soil characteristics on this selection could be examined by comparing the diversities of the populations of fluorescent pseudomonads associated with the roots of a plant species cultivated in two soils sterilized and then supplemented with the same populations of fluorescent pseudomonads prior to the plant cultivation. Our results indicate that research on rhizosphere-competent fluorescent pseudomonads should take into consideration not only the host plant but also the soil characteristics.

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