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Siderophore Typing, a Powerful Tool for the Identification of Fluorescent and Nonfluorescent Pseudomonads

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A total of 301 strains of fluorescent pseudomonads previously characterized by conventional phenotypic and/or genomic taxonomic methods were analyzed through siderotyping, i.e., by the isoelectrophoretic characterization of their main siderophores and pyoverdines and determination of the pyoverdine-mediated iron uptake specificity of the strains. As a general rule, strains within a well-circumscribed taxonomic group, namely the species *Pseudomonas brassicacearum*, *Pseudomonas fuscovaginae*, *Pseudomonas jessenii*, *Pseudomonas mandelii*, *Pseudomonas monteilii*, "*Pseudomonas mosselii*," "*Pseudomonas palleronii*," *Pseudomonas rhodesiae*, "*Pseudomonas salomonii*," *Pseudomonas syringae*, *Pseudomonas thivervalensis*, *Pseudomonas tolaasii*, and *Pseudomonas veronii* and the genomospecies FP1, FP2, and FP3 produced an identical pyoverdine which, in addition, was characteristic of the group, since it was structurally different from the pyoverdines produced by the other groups. In contrast, 28 strains belonging to the notoriously heterogeneous *Pseudomonas fluorescens* species were characterized by great heterogeneity at the pyoverdine level. The study of 23 partially characterized phenotypic clusters demonstrated that siderotyping is very useful in suggesting correlations between clusters and well-defined species and in detecting misclassified individual strains, as verified by DNA-DNA hybridization. The usefulness of siderotyping as a determinative tool was extended to the nonfluorescent species *Pseudomonas corrugata*, *Pseudomonas frederiksbergensis*, *Pseudomonas graminis*, and *Pseudomonas plecoglossicida*, which were seen to have an identical species-specific siderophore system and thus were easily differentiated from one another. Thus, the fast, accurate, and easy-to-perform siderotyping method compares favorably with the usual phenotypic and genomic methods presently necessary for accurate identification of pseudomonads at the species level.

Siderophores are molecules especially designed to trap traces of iron(III) under the form of very stable complexes (48). They are excreted by iron-starved microorganisms, and after the complexes have formed, they are internalized into the cells by specific membrane receptors (36). Most microorganisms, including fungi and bacteria, use siderophores to fulfill their iron requirements, and a couple of hundred of different siderophore structures have been described (48).

Pyoverdine, the well-known yellow-green fluorescent pigment characteristic of the fluorescent *Pseudomonas* species (15), is the major siderophore of these bacteria (27, 28). In the structure of pyoverdine, there is a quinoleinic chromophore which imparts the color and fluorescence to the molecule, associated with a peptide chain of L-, D-, and uncommon amino acids, such as ∂-*N*-hydroxyornithine and β-hydroxyaspartic acid (9). Both parts of the molecule participate in the complexation of the iron(III) ion, as illustrated in Fig. 1 for the pyoverdine of *P. aeruginosa* ATCC 27853 (44).

The remarkable diversity of pyoverdines is illustrated by the close to 50 different molecules which have been identified, differing from one another by the number (6 to 12) and types of amino acids in the peptide chain (18). Several pyoverdine molecular forms differing in the acyl chain attached to the chromophore (see Fig. 1) but having identical peptide chains may coexist in the growth supernatant of a single strain. The nature of the acyl chain does not affect iron transport (30), and these variant molecules represent isoforms of an otherwise identical pyoverdine. The diversity at the level of peptide structures confers the strict specificity of recognition usually observed between a given strain and its pyoverdine (21). Exceptions to this rule could be due to the presence of multiple receptors in a given strain (24) or to identical receptor recognition sites shared by several pyoverdines (19, 29, 33).

The main question that guided the present work was to determine the correlation between the structural diversity of pyoverdines, as reflected by isoelectrophoresis patterns and iron transport specificity, and the taxonomic diversity and heterogeneity among the fluorescent *Pseudomonas* species.

The genus *Pseudomonas* was defined by Migula in 1894 (34). Since then, its taxonomy has undergone many changes, with, as first attempts, the proposal of keys of little value such as the rod shape, negative Gram staining, and motility by means of polar flagella (14). An extensive phenotypic characterization of strains (42), mainly DNA-DNA hybridization, rRNA-DNA hy-

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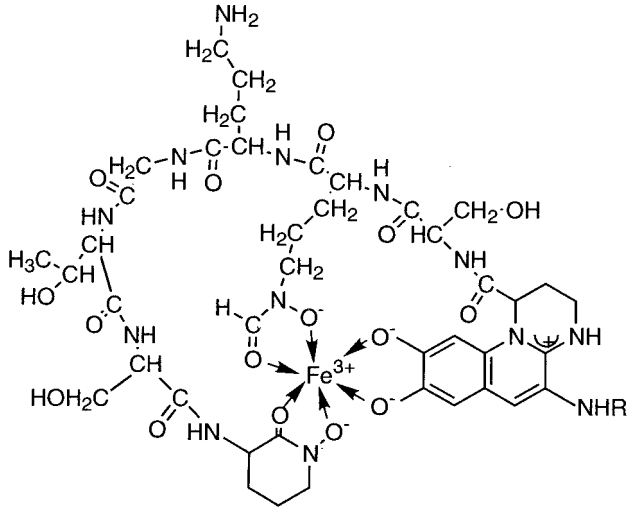

FIG. 1. Structure of the iron complex of the *P. aeruginosa* ATCC 27853 pyoverdine. The acyl chain (R group) could be succinic acid, succinamide, or glutamic acid (44).

bridization, and 16S rDNA sequencing, resulted later on in a clear demonstration that the genus *Pseudomonas* was phylogenetically very complex and could be subdivided into a number of taxa at the genus level (4, 22, 38). The name *Pseudomonas* is now reserved for RNA homology group I, which includes the type species, *Pseudomonas aeruginosa*, while the members of the other rRNA groups are assigned to various other genera (4, 22).

Both phenotypic and genomic approaches were essential components of what was to be known as polyphasic taxonomy (46), which allowed the recent description of numerous new species (1, 3, 6, 13, 16, 17, 37, 43, 47). The present work has focused mainly on fluorescent *Pseudomonas* strains, including the new species and numerous phenotypic clusters under investigation, and extended to some well-defined nonfluorescent species of the genus, showing that a precise taxonomic allocation which parallels the polyphasic taxonomy data can be simply achieved by siderotyping. The procedures described in this communication fulfill the demand for fast and reliable species identification. Moreover, the techniques may be expanded to solve identification of numerous siderophore-producing microbial groups other than the *Pseudomonas* species.

MATERIALS AND METHODS

Bacterial strains. The fluorescent pseudomonads (301 strains) included four collection strains and 297 natural isolates of various origins (61 isolates from soils, 96 from water, 131 from plant environments, and 9 from clinical specimens) grouped according to the taxonomic investigations previously done in the authors' laboratories into 13 species, 3 genomospecies (i.e., group of bacteria which show ≥70% DNA relatedness), and 23 phenotypic clusters (i.e., groups of bacteria which clustered in numerical phenotypic analysis) (Table 1). The strains identified as *P. fluorescens* biovar I in Table 1 are considered a phenotypic group because their assignation has only been obtained through a conventional limited number of phenotypic characteristics (8). Most of the species and genomospecies in Table 1 have already been described (1, 13, 16, 17, 43, 47). Three of them, "*P. mosselii*," "*P. palleronii*," and "*P. salomonii*," will soon be published. As detailed individually in Table 1, the bacteria belonging to these species have been subjected to a polyphasic taxonomic approach, including a numerical phenotypic analysis and several genomic methods, with percent G+C determination, 16S

TABLE 1. Bacterial strains

Taxonomic group	Origin	No. of strains	Taxonomic investigations[a]	Source[b] or reference
Fluorescent pseudomonads				
Pseudomonas brassicacearum	Plant rhizosphere	10	b, d, e, f, g	1
Pseudomonas fluorescens biovar I	Plant environment	24	a	CFBP
	Culture collections	4	a	ATCC, CIP
Pseudomonas fuscovaginae	Rice	13	b, e, f	CFBP
Pseudomonas jessenii	Mineral water	8	b, d, e, f	47
Pseudomonas mandelii	Mineral water	25	b, d, e, f	47
Pseudomonas monteilii	Clinical specimens	9	a, b, c, e, f	17
"*Pseudomonas mosselii*"	Mineral water	11	a, b, d, e, f	CFML
"*Pseudomonas palleronii*"	Rice	11	b, d, e, f	CFBP
Pseudomonas rhodesiae	Mineral water	7	b, e, f	13
"*Pseudomonas salomonii*"	Garlic	15	b, d, e, f	CFBP
Pseudomonas syringae	Plant	26	c, d, e, f, g	CFBP
Pseudomonas thivervalensis	Plant rhizosphere	6	b, d, e, f, g	1
Pseudomonas tolaasii	Mushroom caps	8	b	CFBP
Pseudomonas veronii	Mineral water	8	b, e, f	16
Genomospecies FP1, FP2, FP3	Plant	18	b, e, f	43
Clusters XXXIIb and XXXVb	Mineral water	37	b	CFML
Clusters D1 to D12	Soil	32	a, b, h	26
Clusters C1 to C9	Soil	29	a, b, c, h	25
Nonfluorescent pseudomonads				
Pseudomonas corrugata	Plant	13	b, e, i, j	43
Pseudomonas frederiksbergensis	Soil	5	b, d, e, f	3
Pseudomonas graminis	Plant	19	a, d, e, f, g, j	6
Pseudomonas plecoglossicida	Fish	6	a, d, e, f	37

[a] a, phenotyping (8); b, numerical phenotypic taxonomy; c, ribotyping; d, 16S ribosomal DNA (rDNA) sequencing; e, DNA-DNA hybridization (S1 method plus ΔT_m); f, percent G+C; g, amplified rDNA restriction analysis; h, repetitive extragenic palindromic-PCR genomic fingerprinting, i, lipopolysaccharide pattern; j, fatty acid methyl ester profiles.

[b] Abbreviations: ATCC, American Type Culture Collection, Manassas Va.; CFBP, Collection Française de Bactéries Phytopathogènes, INRA-Angers, France; CFML, Collection de la Faculté de Médecine de Lille, Lille, France; CIP, Collection de l'Institut Pasteur, Paris, France.

rDNA sequencing, and DNA-DNA hybridization as the most frequently used methods.

The 23 phenotypic clusters represented groups of fluorescent pseudomonads which clustered in numerical phenotypic analysis with none of the reference strains conventionally used in such studies and therefore were kept for further taxonomic analysis. A few of them have already been subjected to a restricted number of genomic methods (see Table 1). Forty-three strains of recently described (3, 6, 37, 43) new nonfluorescent *Pseudomonas* species, namely *Pseudomonas corrugata* (13 strains), *Pseudomonas graminis* (19 strains), *Pseudomonas frederiksbergensis* (5 strains), and *Pseudomonas plecoglossicida* (6 strains), were added to the study. *P. plecoglossicida* was first described as a fluorescent species based on a weakly positive King's B test (37). However, none of the iron-poor growth media routinely used for pyoverdine production (CAA medium, succinate medium, and King's B liquid medium) allowed such production in our hands, while another nonfluorescent siderophore was produced in large amounts by the *P. plecoglossicida* strains in such media. Furthermore, no pyoverdine-mediated iron uptake was observed with any of 35 structurally different pyoverdines. Therefore, *P. plecoglossicida* should be considered a nonfluorescent *Pseudomonas* species.

Preservation of the bacteria and culture conditions using iron-poor succinate or Casamino Acids (CAA) medium were done as described previously (31).

Siderophore isoelectrofocusing. The method of Koedam et al. (23) was adapted to the model 111 mini-IEF cell from Bio-Rad. Casting of the gels (125 by 65 by 0.4 mm, made of 5% polyacrylamide containing 2% Bio-Lyte 3-10 ampholines) and isoelectrofocusing (IEF) were performed according to the manufacturer's recommendations [a three-step electrophoresis (15 min at 100 V, 15 min at 200 V, and 1 h at 450 V) run at 4°C]. Deposits on the gel were done as 1 μl of 20-fold-concentrated CAA culture supernatants (concentration through lyophilization). Immediately after electrophoresis, the pyoverdine bands were visualized under UV at 365 nm, and their isoelectric points (pI) were determined by using an internal standard containing a mix of known purified pyoverdines (18).

Siderophores produced by the nonfluorescent pseudomonads were detected in the growth culture supernatants by the Chrome Azurol S (CAS) reagent (41) and visualized on the IEF gels by the CAS overlay method (23). Repeated experiments with different gels and different ampholine commercial samples demonstrated a standard deviation of ±0.1 for pI values above pH 6.0 and ±0.2 for pI values below pH 6.0. Therefore, the expected identity in siderophore-IEF profiles was controlled by performing comigration of the siderophores on the same gel.

Siderophore-mediated iron uptake. Purification of pyoverdines, preparation of the [^{59}Fe]pyoverdine complexes, and the uptake experimental conditions have been described recently (18, 35). Briefly, bacterial cells from 40 h of culture in succinate medium were harvested by centrifugation, washed once with distilled water, and resuspended at an optical density at 600 nm of 0.33 in an incubation medium made of succinate medium with the nitrogen source omitted. Label mix containing [^{59}Fe]pyoverdine complex consisted of 5 μl of the commercial $^{59}Fe^{3+}$ solution (iron chloride in 0.1 M HCl, specific activity 110 to 925 MBq/mg of iron; Amersham) diluted first with 100 μl of water and then mixed with 10 μl of a 6.5-mg/ml XAD-purified pyoverdine solution. The final volume of the label mix was adjusted after 30 min of incubation at room temperature to 1 ml with incubation medium.

The pyoverdines listed in Table 3 were used as well as a collection of 35 structurally known pyoverdines used as references for siderovar assignation (see reference 35 for a complete listing). Bacterial suspension (1.8 ml) was mixed at time zero with 0.2 ml of label mix. After 20 min of incubation with gentle shaking in a waterbath at 25°C, 1 ml of the bacterial suspension was rapidly filtered through a Whatman nitrocellulose filter (0.45-μm porosity), and the filter was washed twice with 2 ml of fresh incubation medium. Each filter was then wrapped in aluminum foil, and counts were determined in a Gamma 4000 counter (Beckman). The radioactivity in the remaining 1 ml of bacterial suspension was counted directly to determine the total amount of radioactivity present in the assay. Control assays without bacteria were performed to verify the complete solubility of labeled iron through pyoverdine complexation.

Siderophore-mediated iron uptake in strains belonging to the nonfluorescent *Pseudomonas* species was determined in the same way but with a label mix made with siderophore-containing culture supernatant (100 μl). Assays without cells ensured that the siderophore content was high enough to reach a full complexation of the labeled iron. Uptake data expressed in tables and figures are average values of at least two independent experiments.

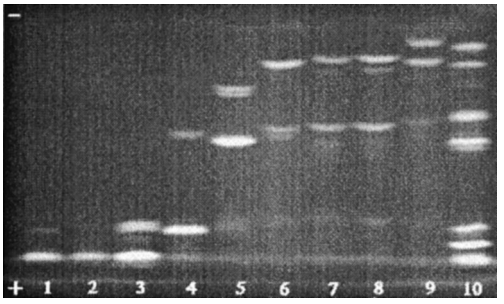

FIG. 2. Isoelectrophoretic patterns of the pyoverdine isoforms produced by (from left to right) *P. syringae* ATCC 19310^T, *P. syringae* CFBP 2106, strain C-TR1015 (cluster C9), *P. monteilii* CFML 90-54, *P. rhodesiae* CFML 92-104, *P. tolaasii* CFBP 2068^T, *P. mandelii* CFML 95-303^T, strain CFML 96-338 (cluster XXXIIb), the strain CFML 96-299 (cluster XXXIIb). The last lane contained an internal standard for pI measurement (18).

RESULTS

Definition of siderotypes according to pyoverdine isoelectrofocusing profiles. A first set of experiments consisted in analyzing by IEF the pyoverdines produced by iron-starved bacteria of the different taxonomic groups. As shown in Fig. 2, one to three major isoforms and a few supplementary minor bands for some strains were defined for each pyoverdine-containing growth supernatant. The determination of the corresponding pI for each band and its representation on a pH scale as a bar with thickness related to the intensity of fluorescence allowed the characterization of numerous pyoverdine-IEF patterns, as represented in Table 2. A comparison of the IEF patterns led to the following observations.

(i) Strains belonging to the same group usually developed an identical pyoverdine-IEF pattern. This is illustrated in Fig. 2, lanes 1 and 2, for two *P. syringae* isolates, for which a main acidic pyoverdine band was observed together with a weaker upper band. Complete data are given for each group in Table 2, with the number of strains having the same IEF pattern over the total number of strains analyzed within each group given. The few missing strains within a few groups were isolates naturally defective in pyoverdine production and consequently not typeable by isoelectrofocusing. Two exceptions concerning one pyoverdine-producing strain in cluster XXXIIb and another one in cluster XXXVb will be discussed below.

(ii) A majority of the pyoverdine-IEF patterns were well differentiated from one another by the position (pI), number, and intensity of the pyoverdine bands (Table 2). The 13 species were each characterized by an original pyoverdine-IEF profile, while strains belonging to clusters or genomospecies could be accommodated within 10 different IEF patterns. It should be stated that most of the clusters or genomospecies having an identical IEF pattern (e.g., the genomospecies FP1 and FP2 and the soil bacteria in clusters C4 and C5 and in clusters D2 to D8) had their origin in the same collection. Therefore, these clusters or genomospecies were grouped together in Table 2 according to their IEF pattern.

Groups originating from separate collections and not differentiated by IEF were, as shown in Table 2, the genomospecies FP3 and *P. rhodesiae*, the clusters D2 to D8 and *P. jessenii*, the clusters C1 to C3, C8, D9 to D11, and *P. brassicacearum*, and

TABLE 2. Pyoverdine isoelectrophoretic patterns characterizing *Pseudomonas* species, genomospecies, and phenotypic clusters

Taxonomic group	Pattern no.	Reference strain	No. of strains with profile/no. tested	pH 4 5 6 7 8 9
Cluster D1	1	D-TR133	5/5	
Cluster D12	2	D-LE411J	2/2	
Pseudomonas syringae	3	ATCC 19310^T	26/26	
"*Pseudomonas salomonii*"	4	CFBP 2022	15/15	
Pseudomonas fuscovaginae	5	CFBP 2065	12/13	
Pseudomonas tolaasii	6	CFBP 2068^T	8/8	
Pseudomonas monteilii	7	CFML 90-54	7/9	
"*Pseudomonas mosselii*"	8	CFML 90-83	10/11	
Pseudomonas thivervalensis	9	CFBP 11864	6/6	
Genomospecies FP3	10	CFBP 10569	3/3	
Pseudomonas rhodesiae	11	CFML 92-104	7/7	
Cluster C6	12	C-LR711	6/6	
Cluster C7	13	C-TRP112	5/5	
Clusters D2–D8	14	D-S1026	17/17	
Pseudomonas jessenii	15	CFML 95-307^T	8/8	
"*Pseudomonas palleronii*"	16	CFBP 4390	10/11	
Pseudomonas veronii	17	CFML 92-124	8/8	
Cluster XXXVb	18	CFML 96-328	7/8	
Genomospecies FP1 and FP2	19	CFBP 10551	15/15	
Clusters C1–C3, C8, C9, D9–D11	20	C-TR1015	20/22	
Pseudomonas brassicacearum	21	CFBP 11874	10/10	
Clusters C4 and C5	22	C-S111	4/4	
Pseudomonas mandelii	23	CFML 95-303^T	25/25	
Cluster XXXIIb	24	CFML 96-338	28/29	

the clusters C4 C5, XXXIIb, and *P. mandelii* (illustrated in Fig. 2, lanes 7 and 8, for *P. mandelii* CFML 96-303^T and strain CFML 96-338 of cluster XXXIIb, respectively). Thus, a total of 18 different IEF profiles were found among the 13 species and 26 clusters or genomospecies in Table 2.

Definition of siderotypes according to pyoverdine-mediated iron uptake. Identical IEF patterns usually correspond to identical pyoverdines (10, 32), but they could also correspond to pyoverdines presenting structural differences not affecting the global charge of the molecule. The usually strict specificity of pyoverdines in iron transport (21) should, however, allow an easy discrimination between identical and structurally different pyoverdines. Therefore, the pyoverdine-IEF analysis was supported with a second set of tests designed to determine the specificity of the pyoverdine-mediated iron uptake. One strain of each IEF siderotype, as defined in Table 2, was selected, and its pyoverdine was purified (9, 28). The uptake ability of the producing strain and of the other strains belonging to the same group was tested using the [^{59}Fe]pyoverdine complex, starting from the purified compound. Moreover, to test the pyoverdine identities between groups as revealed by IEF, the pyoverdines isolated from one representative of each group were tested in cross-uptake experiments involving the 24 producing strains.

Results can be summarized as follows. (i) Apart from one pyoverdine-deficient *P. monteilii* strain, all strains within the same group and having an identical siderotype efficiently assimilated iron complexed to the corresponding pyoverdine (data not shown). The results thus confirmed the identity in structure of the pyoverdines produced by strains of the same group, as shown by the IEF data. Moreover, all the pyoverdine-deficient isolates except the *P. monteilii* isolate cited above showed efficient iron incorporation towards the pyoverdine characteristic of their group and were thus confirmed in their respective clusters.

(ii) Depending on the strains, three types of responses were observed in the cross-iron uptake experiments. Five groups, corresponding to pyoverdines 1 to 3, 6, and 7 (Table 3), were characterized by a strict specificity of recognition towards their own pyoverdine. Another set, corresponding to pyoverdines 4, 5, 8, and 9, demonstrated the highest uptake efficiency towards

TABLE 3. ^{59}Fe incorporation in the 24 IEF groups of fluorescent pseudomonads as mediated by their pyoverdines

Group	Pyoverdine no.	^{59}Fe uptake[a] 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Cluster D1	1	
Cluster D12	2	
Pseudomonas syringae	3	
"*Pseudomonas salomonii*"	4	
Pseudomonas fuscovaginae	5	
Pseudomonas tolaasii	6	
Pseudomonas monteilii	7	
"*Pseudomonas mosselii*"	8	
Pseudomonas thivervalensis	9	
Genomospecies FP3	10	
Pseudomonas rhodesiae	11	
Cluster C6	12	
Cluster C7	13	
Clusters D2-8	14	
Pseudomonas jessenii	15	
"*Pseudomonas palleronii*"	16	
Pseudomonas veronii	17	
Cluster XXXVb	18	
Genomospecies FP1, FP2	19	
Clusters C1-3, C8, C9, D9-11	20	
Pseudomonas brassicacearum	21	
Clusters C4, C5	22	
Pseudomonas mandelii	23	
Cluster XXXIIb	24	

[a] Black square, homologous incorporation (efficiency recorded at 100%); dark grey square, heterologous incorporation, with 82 to 113% efficiency compared to the homologous system; light grey square, heterologous incorporation, with 8 to 40% efficiency; white square, no incorporation.

their own pyoverdine but were able to use some other pyoverdines, albeit at much lower efficiency (8 to 40% of the homologous system). Finally, other groups displayed a maximal efficiency of iron uptake for their own pyoverdine but also for one or two heterologous pyoverdines with, for some of them, supplementary cross-reactions at lower efficiency. The reciprocity of recognition for groups presenting high cross-incorporation efficiency favors the hypothesis that close pyoverdine structure relationships must exist between these groups. A few of them (groups 12, 13, 16, and 19, Table 2) displayed a specific pyoverdine-IEF pattern compared to their cross-reacting counterparts (Table 3), strongly suggesting structurally different pyoverdines within these groups.

As already suspected for some fluorescent pseudomonads (19, 29) and recently demonstrated for two strains belonging to clusters C6 and C7 (33), such cross-reactivity for pyoverdines that are different in structure is related to the presence of an identical motif likely acting as the outer membrane ferripyoverdine receptor recognition site within the otherwise different peptide chains. From the comparison of the pyoverdine-IEF profiles of the other cross-reacting groups (Table 2, groups 10 and 11, 14 and 15, 17 and 18, 20 and 21 or 22, and 23 and 24), it is evident that cross-reactivity between these groups is relevant to the identity of the pyoverdines. Thus, some of the phenotypic groups could be combined with well-defined species in so-called siderovars, i.e., groups of strains having an identical pyoverdine system for iron uptake, as attested by an identity in pyoverdine-IEF pattern and highly efficient pyoverdine-mediated iron cross-incorporation.

Correlation between siderovar and bacterial species. As described in Table 2, all strains representative of a given cluster usually produced identical pyoverdines. As already stated, some strains corresponding to pyoverdine-deficient isolates were observed within a few clusters. These deficient strains were therefore typed through uptake measurements as mentioned above. The exceptions relevant to cluster XXXIIb and cluster XXXVb, however, refer within each cluster to one isolate (CFML 96-299 for cluster XXXIIb and CFML 97-468 for cluster XXXVb) which did produce pyoverdine, but of a different IEF profile than the one displayed by all the other strains of the cluster (illustrated in Fig. 2, lanes 8 and 9, for CFML 96-299 and for another strain of cluster XXXIIb, respectively).

Therefore, strains belonging to these two clusters, including the ones with a particular pyoverdine-IEF behavior, were analyzed for their genomic homogeneity by DNA-DNA hybridization. The type strain of *P. mandelii* (strain CFML 95-303^T) was included in the study relevant to cluster XXXIIb because pyoverdine-IEF (Table 2) and cross-uptake studies (Table 3) indicated that the two groups belonged to the same siderovar. For the same reasons, the type strain of *P. veronii* (CFML

TABLE 4. DNA-DNA hybridization, pyoverdine (PVD) isoelectric profiles, and PVD-mediated iron uptake capacities of strains relevant to phenotypic cluster XXXIIb and *P. mandelii* 95-303^T

Phenotypic cluster or species	CFML strain no.	% DNA hybridization with CFML 96-338 DNA	pIs of PVD isoforms	Iron uptake[a] mediated by PVD from CFML strains		
				96–338	95–303	96–299
P. mandelii	95-303^T	86	8.9, 8.7, 7.4	103	100	0
XXXIIb	96-299	44	9.2, 8.8, 7.5	0	0	100
XXXIIb	96-311	91	8.9, 8.7, 7.4	92	95	0
XXXIIb	96-330	77	8.9, 8.7, 7.4	102	110	0
XXXIIb	96-337	88	8.9, 8.7, 7.4	99	104	0
XXXIIb	96-338	100	8.9, 8.7, 7.4	100	101	0
XXXIIb	96-349	77	8.9, 8.7, 7.4	102	105	0
XXXIIb	96-350	90	8.9, 8.7, 7.4	87	96	0
XXXIIb	97-394	95	8.9, 8.7, 7.4	98	106	0

[a] Expressed as a percentage of the iron incorporation in the homologous system.

92-134^T) was included with cluster XXXVb in the DNA-DNA hybridization experiments.

Table 4 clearly shows that all strains of the phenotypic cluster XXXIIb included in the study, except strain CFML 96-299, formed a homogeneous genomic cluster, with DNA-DNA hybridization values not below 77% with respect to the reference strain (CFML 96-338). *P. mandelii* CFML 96-303^T DNA hybridized at 86% with the reference strain DNA, a result which allowed the assignment of strains of cluster XXXIIb to the species *P. mandelii*, except strain CFML96-299, which, because of a low DNA-DNA hybridization value (44%), had to be removed from the genomic cluster. Pyoverdine-mediated iron uptake data fully supported such conclusions because all strains of cluster XXXIIb (except CFML 96-299) and the *P. mandelii* type strain were able to incorporate the iron chelated by pyoverdine(96-338) or by the pyoverdine of *P. mandelii* at identical efficiency. Conversely, these strains were unable to use as an iron transporter the pyoverdine of strain CFML 96-299 (Table 4).

Similar data were obtained for strains of cluster XXXVb and *P. veronii* CFML 92-134^T. DNA-DNA hybridization values within this cluster ranged from 70 to 100% (with a ΔT_m of 2°C for the lowest value), except for strain CFML 97-468, which, on account of a 30% DNA-DNA hybridization value, was excluded from the genomic group. This strain, whose pyoverdine-IEF profile was original compared to that of the other strains in cluster XXXVb and *P. veronii* (pI values of 7.4 and 5.2, respectively, instead of 8.4, 7.5, and 5.2 for the others), was the only one which did not incorporate the ferripyoverdines of the genomic XXXVb group and of *P. veronii* CFML 92-134^T. Moreover, and in agreement with the siderotyping data, all strains of the genomic cluster XXXVb should be assigned to *P. veronii,* since the type strain of this species hybridized the DNA of the XXXVb reference strain with a value of 76% (ΔT_m, 1°C).

These data clearly illustrate the power and accuracy of siderotyping for bacterial identification and species assignment, since it allowed conclusions identical to those obtained by DNA-DNA hybridization.

Absence of correlation for the poorly defined *P. fluorescens* species. The pioneering work on *Pseudomonas* taxonomy of Stanier, Palleroni, and Doudoroff (42) was based exclusively on phenotypical characterization of bacteria and resulted in the recognition of major taxonomic groups ranked at the species level, such as *P. aeruginosa*, *P. putida*, and *P. fluorescens*. Some phenotypic heterogeneity, however, was implicitly recognized, with the still well used division of some species into biovars, i.e., biovars A and B for *P. putida* and biovars I to VI for *P. fluorescens*. Although no extensive polyphasic taxonomic characterization within these species is presently available, their taxonomic heterogeneity has been strongly suggested in some cases by numerical taxonomy and genomic studies (8, 20, 22, 49).

The siderotyping analysis performed in the present work on 28 strains (4 collection strains and 24 natural isolates) ascribed to *P. fluorescens* biovar I according to the limited panel of general phenotypic properties conventionally used (8) clearly showed that the correlation between siderovar and species, as established above, was not respected for *P. fluorescens*. As shown in Table 5, 10 different pyoverdine-IEF patterns were recognized among these strains. Some patterns were identical to those described (Table 2) for the species *P. rhodesiae* (three strains) *P. mandelii* (two strains), and *P. jessenii* (one strain). These correlations were confirmed by cross-uptake (data not shown). The most frequent IEF pattern was the one characterizing the pyoverdine of the type strain of the species, *P. fluorescens* ATCC 13525, also found for eight strains of different origins and belonging to different collections.

Other correlations were obtained with structurally known pyoverdines produced by as yet very poorly characterized fluorescent pseudomonads: five strains could be assigned to the same siderovar as *P. fluorescens* 18.1 (2), four strains to the siderovar of *Pseudomonas* sp. strain B10 (45), and one strain to the siderovar of *Pseudomonas* sp. strain A214 (11). Three strains each had a specific pyoverdine-IEF pattern and did not recognize any of the members of a collection of 35 pyoverdines of known structure used as references for siderovar assignation. Therefore, these strains remained unclassified at the siderovar level. Obviously, the multiplicity of siderovars among *P. fluorescens* biovar I parallels the taxonomic heterogeneity recognized for the so-called *P. fluorescens* strains and shows that heterogeneity already exists at the biovar level.

Nonfluorescent *Pseudomonas* species. Some nonfluorescent *Pseudomonas* species, including *P. corrugata*, *P. graminis*, *P. frederiksbergensis*, and *P. plecoglossicida*, have recently been described based on phenotypic as well as genomic taxonomic studies (3, 6, 37, 43). Apart from one strain of *P. graminis*, all strains produced siderophores, since they reacted positively

TABLE 5. Siderovar assignation of *P. fluorescens* biovar I strains of various origins

P. fluorescens bv. I strain(s)	Origin	pI of pyoverdine isoforms	Siderovar assignation according to IEF and uptake
CFBP 11347, 11355, 11356 ATCC 17563	Corn Sputum	4.8, 7.2, 8.4	*P. fluorescens* 18.1
CFBP 11358 CFBP 12206	Corn Salad	5.2, 7.6, 8.7, 9.0	*P. mandelii*
CFBP 11354	Corn	7.5, 9.0	Undetermined
CFBP 11343, 11344 CFBP 11400 CFBP 12180	Corn Soybean Salad	5.1, 7.4	*Pseudomonas* sp. strain B10
CFBP 11365	Corn	7.8, 9.0, 9.2	*P. jessenii*
CFBP 12147, 12152, 12155, 12204 CFBP 11345, 11351 ATCC 13525, CIP 73.25 CIP 56.90	Salad Corn Water Egg	7.2, 8.6, 8.7	*P. fluorescens* ATCC 13525^T
CFBP 11363 CFBP 12156	Corn Salad	7.5, 8.4, 8.5	Undetermined
CFBP 12297, 12299, 12300	Sunflower	7.4, 8.3, 8.4	*P. rhodesiae*
CFBP 12298	Sunflower	7.2, 7.3, 8.3, 8.6	Undetermined
CFBP 11357	Corn	3.9, 5.0, 5.3	*Pseudomonas* sp. strain A214

with the CAS reagent (41) when grown under iron starvation. The siderophores present in the growth supernatants were subjected to IEF and were revealed on the electrophoresed gels by the CAS overlay method (23). The siderophore-producing strains belonging to a given species were each characterized by a single CAS-reacting spot which presented the same pI value for all the strains (Table 6), strongly suggesting that all strains of a given species were producing an identical siderophore. The pI value differed from one species to another, demonstrating that, as for the fluorescent pseudomonads, the nature of the siderophore should be species specific among the four species analyzed.

Siderophore identity and siderophore specificity were also tested by iron uptake studies performed on these strains and their siderophores. As indicated in Table 6, all *P. graminis* strains, including the isolate which was CAS negative because of a lack of siderophore production, were able to incorporate the siderophore produced by any strain of the same species at an identical efficiency. Similar results were obtained for the three other species and their strains and siderophores (Table 6). Moreover, heterologous uptake involving one strain of a given species and the siderophores of the other species showed no cross-incorporation (data not shown).

DISCUSSION

For the purpose of rapid identification, nucleic acid methods are lengthy and cumbersome, and the phenotypic heterogeneity of many groups limits the usefulness of most phenotypic tests when used individually. Even though the siderotype is essentially a phenotypic characteristic, the fact that all the strains of a given natural group should belong to the same siderotype appears to be unique. As we have mentioned, as a general rule, all strains belonging to a well-defined genomic group produce an identical pyoverdine, and each genomic group is characterized by a specific pyoverdine. As also shown, identical conclusions are valid for nonfluorescent *Pseudomonas* species and their siderophores.

Perhaps an explanation for these facts may be found in the fundamental role that the siderophores play in microbial nutrition. Iron is absolutely required in the aerobic metabolism of pseudomonads. However, in spite of its abundance, the assimi-

TABLE 6. Siderophore production by strains belonging to nonfluorescent *Pseudomonas* species

Bacterial species	No. of producing strains/no. tested	Siderophore produced	Siderophore pI	Siderophore-mediated ^{59}Fe incorporation
Pseudomonas corrugata	13/13	Corrugatin[a]	5.0	+, all strains
Pseudomonas plecoglossicida	6/6	Unknown	7.2	+, all strains
Pseudomonas graminis	18/19	Unknown	5.9	+, all strains
Pseudomonas frederiksbergensis	5/5	Unknown	≤3.9	+, all strains

[a] As evidenced by comparison with the siderophore produced by another *P. corrugata* strain described previously (40).

latable form of this element is occasionally very scarce due to its low solubility in aerobic environments. These factors may have imposed strong selective pressures resulting in the development of specific mechanisms for iron complexation and utilization, and in the competition for survival, selection must have favored those organisms capable of synthesizing siderophores combined with utilization of the iron complexes. It is quite possible, therefore, that this type of response to the challenge of surviving in environments of low assimilatable iron content may have taken part very early in the differentiation of prokaryotic species during evolution. This could explain what is particularly highlighted in the present study, that pyoverdine, the siderophore of the fluorescent pseudomonads, represents a very powerful taxonomic marker.

It should be emphasized that numerous clusters or species included in the present study comprised bacteria that had their origins in different collections of strains resulting from work in different laboratories. Consequently, they have not been compared until the present time. The siderotyping data indicated that some of them, those belonging to the same siderovar, could be taxonomically closely related, if not identical. Some preliminary data already favored such a hypothesis because an identical ribotyping profile has been observed for strains belonging to genomospecies FP3 and *P. rhodesiae* (L. Gardan and P. Grimont, unpublished data). Moreover, the perfect correlation between DNA-DNA hybridizations and siderotyping data, as shown in the present study for cluster XXXIIb and *P. mandelii* and for cluster XXXVb and *P. veronii*, demonstrates the power of the siderotyping method in bacterial identification.

The results reported on a collection of *P. fluorescens* biovar I strains support this conclusion. These strains have been classified on the basis of a restricted panel of phenotypic characters only, and therefore, their taxonomic heterogeneity is highly suspect (8, 20, 22, 49). Siderotyping clearly established that the collection includes strains whose siderotypes appeared to be identical to those of the recently described species *P. mandelii*, *P. jessenii*, and *P. rhodesiae*. Whether the other siderotypes of biovar I that do not fit with any of the known types correspond to species not yet identified is a question that hopefully will be clarified in time.

The possibility of constructing a genealogic tree of cross-reactive pyoverdines based on the similarities in structure of their peptide part is presently under study. If this project were to be successful, the predictive capacity of siderotyping and its use in phylogenetic studies might be a useful supplement to its already remarkable identification value. Moreover, from preliminary investigations done on rhizobia and *Burkholderia* spp. (12, 28), it appears that the method could be extended to the identification of other siderophore-producing microorganisms. The ability to discriminate among species of enterobacteria or mycobacteria through siderophores has already been reported (5, 7, 39), and a correlation between DNA hybridization groups and siderophore production has been established for *Aeromonas* spp. (50).

In closing, it should be emphasized that siderotyping allowed a correct grouping of the 344 strains analyzed in the present study within a few weeks, while the same results would have required a considerably longer time and greater expense with classical polyphasic taxonomic methods.

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ADDENDUM IN PROOF

It has been suggested that the bacterial group described in the present paper, *Pseudomonas palleronii*, be reassigned as the novel species *Pseudomonas palleroniana* (L. Gardan, P. Bella, J.-M. Meyer, R. Christen, P. Rott, W. Achouak, and R. Samson, Int. J. Syst. Evol. Microbiol, in press). Moreover, the group *Pseudomonas mosselii* has also been recently described (F. Dabboussi, M. Hamze, E. Singer, V. Geoffroy, J. M. Meyer, and D. Izard, Int. J. Syst. Evol. Microbiol. **52:**363–376, 2002).

REFERENCES

1. **Achouak, W., L. Sutra, T. Heulin, J.-M. Meyer, N. Fromin, S. Degreave, R. Christen, and L. Gardan.** 2000. Description of *Pseudomonas brassicacearum* sp. nov. and *Pseudomonas thivervalensis* sp. nov., root-associated bacteria isolated from *Arabidopsis thaliana* and *Brassica napus.* Int. J. Syst. Evol. Microbiol . **50:**9–18.
2. **Amann, C., K. Taraz, H. Budzikiewicz, and J.-M. Meyer.** 2000. The siderophores of *Pseudomonas fluorescens* 18.1 and the importance of cyclopeptidic substructures for the recognition at the cell surface. Z. Naturforsch. **55c:**671–680.
3. **Andersen, S. M., K. Johnsen, J. Sorensen, P. Nielsen, and C. S. Jacobsen.** 2000. *Pseudomonas frederiksbergensis* sp. nov., isolated from soil at a coal gasification site. Int. J. Syst. Environ. Microbiol. **50:**1957–1964.
4. **Anzai, Y., H. Kim, J.-Y. Park, H. Wakabayashi, and H. Oyaizu.** 2000. Phylogenic affiliation of the pseudomonads based on 16S rRNA sequence. Int. J. Syst. Evol. Microbiol. **50:**1563–1589.
5. **Barclay, R., and C. Ratledge.** 1988. Mycobactins and exochelins of *Mycobacterium tuberculosis*, *M. bovis, M. africanum* and other related species. J. Gen. Microbiol. **134:**771–776.
6. **Behrendt, U., A. Ulrich, P. Schumann, W. Erler, J. Burghardt, and W. A. Seyfarth.** 1999. Taxonomic study of bacteria isolated from grasses: a proposed new species *Pseudomonas graminis* sp. nov. Int. J. Syst. Bacteriol. **49:**297–308.
7. **Bosne, S., and V. Levy-Frebault.** 1992. Mycobactin analysis as an aid for identification of *Mycobacterium chelonae* subspecies. J. Clin. Microbiol. **30:** 1225–1231.
8. **Bossis, E., P. Lemanceau, X. Latour, and L. Gardan.** 2000. The taxonomy of *Pseudomonas fluorescens* and *Pseudomonas putida*: current status and need for revision. Agronomie **20:**51–63.
9. **Budzikiewicz, H.** 1997. Siderophores of fluorescent pseudomonads. Z. Naturforsch. Sect. C **52:**713–720.
10. **Budzikiewicz, H., S. Kilz, K. Taraz, and J.-M. Meyer.** 1997. Identical pyoverdines from *Pseudomonas fluorescens* 9AW and from *Pseudomonas putida* 9BW. Z. Naturforsch. Sect. C **52:**721–728.
11. **Buyer, J. S., J. M. Wright, and J. Leong.** 1986. Structure of pseudobactin A214, a siderophore from a bean-deleterious *Pseudomonas*. Biochemistry **25:**5492–5499.
12. **Carson, K. C., J.-M. Meyer, and M. J. Dillworth.** 2000. Hydroxamate siderophores of root nodule bacteria. Soil Biol. Biochem. **32:**11–21.
13. **Coroler, L., M. Elomari, B. Hoste, M. Gillis, D. Izard, and H. Leclerc.** 1996. *Pseudomonas rhodesiae* sp. nov., a new species isolated from natural mineral waters. Syst. Appl. Microbiol. **19:**600–607.
14. **den Dooren de Jong, L. E.** 1926. Bijdrage tot de kennis van Het mineralisatieproces. Nijgh and van Ditmar Uitgevers-Mij, Rotterdam, The Netherlands.
15. **Elliott, R. P.** 1958. Some properties of pyoverdine, the water soluble pigment of the *Pseudomonas.* Appl. Microbiol . **6:**241–246.
16. **Elomari, M., L. Coroler, B. Hoste, M. Gillis, D. Izard, and H. Leclerc.** 1996. DNA relatedness among *Pseudomonas* strains isolated from natural mineral waters and proposal of *Pseudomonas veronii* sp. nov. Int. J. Syst. Bacteriol. **46:**1138–1144.
17. **Elomari, M., L. Coroler, S. Verhille, D. Izard, and H. Leclerc.** 1997. *Pseudomonas monteilii* sp. nov., isolated from clinical specimens. Int. J. Syst. Bacteriol. **47:**846–852.
18. **Fuchs, R., M. Schäfer, V. Geoffroy, and J.-M. Meyer.** 2001. Siderotyping—a powerful tool for the characterization of pyoverdines. Curr. Top. Med. Chem. **1:**31–35.
19. **Georgias, H., K. Taraz, H. Budzikiewicz, V. Geoffroy, and J.-M. Meyer.** 1999. The structure of the pyoverdin from *Pseudomonas fluorescens* 1.3. Structural

and biological relationships of pyoverdins from different strains. Z. Naturforsch. Sect. C **54:**301–308.
20. **Grimont, P. A. D., M. Vancanneyt, M. Lefèvre, K. Vandermeulebroecke, L. Vauterin, R. Brosch, K. Kersters, and F. Grimont.** 1996. Ability of Biolog and Biotype-100 systems to reveal the taxonomic diversity of the pseudomonads. Syst. Appl. Microbiol. **19:**510–527.
21. **Hohnadel, D., and J.-M. Meyer.** 1988. Specificity of pyoverdine-mediated iron uptake among fluorescent *Pseudomonas* strains. J. Bacteriol. **170:**4865–4873.
22. **Kersters, K., W. Ludwig, M. Vancanneyt, P. DeVos, M. Gillis, and K. H. Schleifer.** 1996. Recent changes in the classification of the pseudomonads: an overview. Syst. Appl. Microbiol. **19:**465–477.
23. **Koedam, N., E. Wittouck, A. Gaballa, A. Gillis, M. Höfte, and P. Cornelis.** 1994. Detection and differenciation of microbial siderophores by isoelectric focusing and chrome azurol S overlay. BioMetals **7:**287–291.
24. **Koster, M., W. Ovaa, W. Bitter, and P. J. Weisbeek.** 1995. Multiple outer membrane receptors for uptake of ferric pseudobactins in *Pseudomonas putida* WCS358. Mol. Gen. Genet. **248:**735–743.
25. **Latour, X., T. Corberand, G. Laguerre, F. Allard, and P. Lemanceau.** 1996. The composition of fluorescent pseudomonad populations associated with roots is influenced by plant and soil type. Appl. Environ. Microbiol. **62:**2449–2456.
26. **Lemanceau, P., T. Corberand, L. Gardan, X. Latour, G. Laguerre, J. M. Boeufgras, and C. Alabouvette.** 1995. Effect of two plant species flax (*Linum usitatissinum* L.) and tomato (*Lycopersicon esculentum* Mill.) on the diversity of soil-borne populations of fluorescent pseudomonads. Appl. Environ. Microbiol. **61:**1004–1012.
27. **Meyer, J.-M., and J.-M. Hornsperger.** 1978. Role of pyoverdine$_{Pf}$, the iron-binding fluorescent pigment of *Pseudomonas fluorescens*, in iron transport. J. Gen. Microbiol. **107:**329–331.
28. **Meyer, J.-M., and A. Stintzi.** 1998. Iron metabolism and siderophores in *Pseudomonas* and related species, p. 201–243. *In* T. C. Montie (ed.), Biotechnology handbooks, vol. 10: *Pseudomonas*. Plenum Publishing Co., New York, N.Y.
29. **Meyer, J.-M., A. Stintzi, and K. Poole.** 1999. The ferripyoverdine receptor FpvA of *Pseudomonas aeruginosa* PAO1 recognizes the ferripyoverdines of *P. aeruginosa* PAO1 and *P. fluorescens* ATCC 13525. FEMS Microbiol. Lett. **170:**145–150.
30. **Meyer J.-M., F. Hallé, D. Hohnadel, P. Lemenceau, and H. Ratefiarivelo.** 1987. Siderophores of *Pseudomonas*—biological properties, p. 189–205. *In* G. Winkelmann, D. van der Helm, and J. B. Neilands (ed.). Iron transport in microbes, plants and animals. V.C.H. Verlaggesellschaft, Weinheim, Germany.
31. **Meyer, J.-M., A. Stintzi, D. De Vos, P. Cornelis, R. Tappe, K. Taraz, and H. Budzikiewicz.** 1997. Use of siderophores to type pseudomonads: the three *Pseudomonas aeruginosa* pyoverdine systems. Microbiology **143:**35–43.
32. **Meyer, J.-M., A. Stintzi, V. Coulanges, S. Shivaji, J. A. Voss, K. Taraz, and H. Budzikiewicz.** 1998. Siderotyping of fluorescent pseudomonads: characterization of pyoverdines of *Pseudomonas fluorescens* and *Pseudomonas putida* strains from Antarctica. Microbiology **144:**3119–3126.
33. **Meyer, J.-M., V. A. Geoffroy, C. Baysse, P. Cornelis, I. Barelmann, K. Taraz, and H. Budzikiewicz.** 2002. Siderophore-mediated iron uptake in fluorescent *Pseudomonas*: characterization of the pyoverdine-receptor binding site of three cross-reacting pyoverdines. Arch. Biochem. Biophys. **397:**179–183.
34. **Migula, W.** 1894. Über ein neues System der Bakterien. Arb. Bakteriol. Inst. Karlsruhe **1:**235–238.
35. **Munsch, P., V. Geoffroy, T. Alatossava, and J.-M. Meyer.** 2000. Application of siderotyping for characterization of *Pseudomonas tolaasii* and "*Pseudomonas reactans*" isolates associated with brown blotch disease of cultivated mushrooms. Appl. Environ. Microbiol. **66:**4834–4841.
36. **Neilands, J. B.** 1982. Microbial envelope proteins related to iron. Annu. Rev. Microbiol. **36:**285–309.
37. **Nishimori, E., K. Kita-Tsukamoto, and H. Wakabaiyashi.** 2000. *Pseudomonas plecoglossicida* sp. nov., the causative agent of bacterial haemorrhagic ascites of ayu, *Plecoglossus altivelis*. Int. J. Syst. Evol. Microbiol. **50:**83–89.
38. **Palleroni, N. J., R. Kunisawa, R. Contopoulos, and M. Doudoroff.** 1973. Nucleic acid homologies in the genus *Pseudomonas.* Int. J. Syst. Bacteriol. **23:**333–339.
39. **Reissbrodt, R., and W. Rabsch.** 1988. Further differentiation of *Enterobacteriaceae* by means of siderophore-pattern analysis. Zentbl. Bakteriol. Hyg. A **268:**306–317.
40. **Risse, D., H. Beiderbeck, K. Taraz, H. Budzikiewicz, and D. Gustine.** 1998. Corrugatin, a lipopeptide siderophore from *Pseudomonas corrugata*. Z. Naturforsch. Sect. C **53:**295–304.
41. **Schwyn, B., and J. B. Neilands.** 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. **160:**47–56.
42. **Stanier, R. Y., N. J. Palleroni, and M. Doudoroff.** 1966. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. **43:**159–271.
43. **Sutra, L., F. Sivirio, M. M. Lopez, G. Hunault, C. Bollet, and L. Gardan.** 1997. Taxonomy of *Pseudomonas* strains isolated from tomato pith necrosis: emended description of *Pseudomonas corrugata* and proposal of three unnamed fluorescent *Pseudomonas* genomospecies. Int. J. Syst. Bacteriol. **47:**1020–1033.
44. **Tappe, R., K. Taraz, H. Budzikiewicz, J.-M. Meyer, and J. F. Lefèvre.** 1993. Structure elucidation of a pyoverdin produced by *Pseudomonas aeruginosa* ATCC 27853. J. Prakt. Chem. **335:**83–87.
45. **Teintze, M., and J. Leong.** 1981. Structure of pseudobactin A, a second siderophore from plant growth promoting *Pseudomonas* B10. Biochemistry **20:**6457–6462.
46. **Vandamme, P., B. Pot, M. Gillis, P. De Vos, K. Kersters, and J. Swings.** 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol. Rev. **60:**407–438.
47. **Verhille, S., N. Baida, F. Dabboussi, D. Izard, and H. Leclerc.** 1999. Taxonomic study of bacteria isolated from natural mineral waters: proposal of *Pseudomonas jessenii* sp. nov. and *Pseudomonas mandelii* sp. nov. Syst. Appl. Bacteriol. **22:**45–58.
48. **Winkelmann, G.** 1991. Handbook of microbial iron chelates. CRC Press, Boca Raton, Fla.
49. **Yamamoto, S., and S. Harayama.** 1998. Phylogenetic relationships of *Pseudomonas putida* strains deduced from the nucleotide sequences of *gyrB*, *rpoD*, and 16S rRNA genes. Int. J. Syst. Bacteriol. **48:**813–819.
50. **Zywno, S. R., J. E. L. Arceneaux, M. Altwegg, and B. R. Byers.** 1992. Siderophore production and DNA hybridization groups of *Aeromonas* spp. J. Clin. Microbiol. **30:**619–622.