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Genetic Diversity of *Pseudomonas aeruginosa* Strains Isolated from Patients with Cystic Fibrosis Revealed by Restriction Fragment Length Polymorphism of the rRNA Gene Region

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The restriction fragment length polymorphism patterns of rDNAs from *Pseudomonas aeruginosa* strains isolated from the respiratory tracts of patients suffering from cystic fibrosis were obtained to evaluate the genetic polymorphism of this population of strains. Eighty-seven *P. aeruginosa* strains isolated from 87 patients from diverse areas of France and the ATCC 10145 strain were examined. Four restriction enzymes were used: *Bam*HI, *Cla*I, *Eco*RI, and *Pst*I. Forty-nine strains (56%) were in the three most frequent ribotypes (ribotypes R1 to R3). In addition, hierarchical clustering analysis of the data showed that 71 of the 88 strains (81%) clustered at a distance of less than one-third of the greatest distance observed in the total population. This indicates that clinical isolates implicated in the pathology of cystic fibrosis present a low degree of heterogeneity of rRNA operons, in contrast to the heterogeneity of strains of *P. aeruginosa* isolated from patients with various other pathologies. This relative homogeneity of rRNA genes was observed independently of the clinical status of the patient and the colony morphology.

Pseudomonas aeruginosa is an ubiquitous microorganism widely distributed in soil, water, plants, and the mammalian gut. It has an extraordinary physiological versatility which allows it to adapt to unfavorable surroundings. *P. aeruginosa* from environmental or endogenous sources may be pathogenic for humans, principally patients treated by use of invasive instruments such as catheters or patients who have predisposing factors including infections resulting from burns, malignant diseases, or metabolic disorders. In particular, patients with cystic fibrosis (CF) are susceptible to chronic *P. aeruginosa* infections of the airways, causing pulmonary dysfunction which can lead to death. *P. aeruginosa* is the bacterial species most frequently recovered from the respiratory tracts of chronically infected patients with CF (13). Initially, patients are colonized with a classic *P. aeruginosa* morphotype, and subsequently, adapted mucoid strains become predominant during the evolution of the disease.

The genetic diversity of strains belonging to the species *P. aeruginosa* is probably high (9). The weak linkage disequilibrium between enzyme loci, studied by multilocus enzyme electrophoresis, suggests that there is frequent recombination of chromosomal genes between different strains (22).

Enzyme electrophoretic analysis and other molecular typing methods have shown that the number of clones involved in pathogenic processes is limited to a subset of strains of a species, for example, *Escherichia coli* in neonatal meningitis (23), *Haemophilus influenzae* in neonatal infection (17), and multiresistant *P. aeruginosa* O12 strains in nosocomial outbreaks (7). Similarly, it is possible that strains implicated in pulmonary infections in patients with CF are relatively homogeneous.

Numerous traditional typing methods are based on phenotypic markers which have been recognized as unstable and which are thus not a satisfactory guide to the genetic identity of strains (21). Restriction fragment length polymorphism profiles of rRNA regions (rDNA RFLP patterns) have recently been evaluated and have been shown to be a valuable tool for assessing the genetic diversity of *P. aeruginosa* strains and useful for epidemiological analysis (1, 11, 14).

The objective of the present study was to characterize the genetic polymorphism of a population of *P. aeruginosa* strains isolated from the airways of patients with CF by determination of the rDNA RFLP patterns. We used four endonucleases (*Bam*HI, *Cla*I, *Eco*RI, and *Pst*I) previously used for studies of unrelated *P. aeruginosa* strains to show the high level of heterogeneity of the species (1, 11). The rDNA RFLP data were analyzed in terms of population structure by computation (15).

MATERIALS AND METHODS

Bacterial isolates. Eighty-seven clinical *P. aeruginosa* strains and the ATCC 10145 reference strain were studied (Table 1). They were recovered from the sputum of 87 patients with CF (40 females and 47 males) originating from various regions of France. Eighteen *Staphylococcus aureus*, nine *H. influenzae*, one *Burkholderia cepacia*, and one *Alcaligenes xylosidans* isolate were recovered concomitantly with the *P. aeruginosa* strains. After isolation, the *P. aeruginosa* strains were stored at −80°C in Schaedler-vitamin K_3 broth (bioMérieux, Marcy l'Etoile, France) containing 10% glycerol and were grown on blood agar (bioMérieux) at 37°C in ambient air.

Strains were identified as *P. aeruginosa* according to colony morphology, Gram staining, oxidase reaction, growth at 42°C, ability to produce characteristic pigmentation on King A agar, and the response pattern in the API 20NE system (API-System, La Balme les Grottes, France).

Clinical evaluation. Clinical evaluation was based on a modification of the Schwachman-Kulcyzcki scoring system (5, 20), which involves four clinical elements of evaluation: case history, pulmonary physical findings and cough, growth and nutrition, and chest roentgenogram. For each criterion, the physician assigns a score of 5, 10, 20, or 25 points in accordance with a precise description previously standardized by Doershuk et al. (5). The patients were thereby classified into five categories, as follows: group A, excellent clinical condition, total score of 86 to 100; group B, good clinical condition, total score of 71 to 85; group C, moderate clinical condition, total score of 56 to 70; group D, poor clinical condition, total score of 41 to 55; and group E, weak clinical condition, total score of ≤40.

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TABLE 1. Distribution of morphology, serotype, and ribotype of 87 *P. aeruginosa* strains isolated from patients with CF and the ATCC 10145 reference strain

Patient					Strain		
No.[a]	Sex[b]	Age (yr)	Clinical state[c]	Morphology[d]	Serotype[e]	Ribotype	Ribotype no.
1	F	7	C	R	PAG	B1C1E1P1	1
2	F	9	B	D	NAG	B1C1E1P1	1
3	F	14	D	R	NAG	B7C1E2P7	30
4	M	18	C	G	NAG	B1C3E1P1	3
5	M	7	A	SR	PAG	B1C2E1P1	2
6	M	6	C	D	NAG	B1C1E1P1	1
7	M	18	C	D	NAG	B1C1E1P1	1
8	M	19	D	SR	O6	B1C3E1P1	3
9	M	11	B	S	PAG	B1C2E1P1	2
10	M	7	C	SR	PAG	B1C2E1P1	2
11	M	21	D	R	PAG	B1C3E1P1	3
12	M	7	B	SR	PAG	B1C3E2P1	13
13	M	16	C	SR	NAG	B1C1E1P1	1
14	M	6	B	SR	NAG	B1C1E1P1	1
15	M	7	C	SR	NAG	B1C1E1P1	1
16*	M	11	B	R	PAG	B1C3E1P2	7
17*	F	16	C	SR	PAG	B1C1E1P2	4
18*	M	17	C	SR	PAG	B1C1E1P2	4
19	F	11	C	SR	O1	B1C2E1P1	2
20	F	11	C	D	PAG	B3C2E1P1	25
21	F	18	B	SR	O6	B1C3E1P1	3
22	M	5	E	R	O6	B1C2E1P1	2
23	F	4	D	M	NAG	B3C8E2P7	26
24	F	18	C	SR	PAG	B1C3E1P2	7
25	F	8	C	SR	O3	B1C4E1P2	8
26	F	12	C	SR	PAG	B1C4E1P1	6
27	M	7	B	SR	NAG	B1C4E1P2	8
28	M	12	D	G	NAG	B1C5E1P6	17
29	F	10	C	SR	O6	B1C1E1P1	1
30	M	6	C	D	NAG	B4C4E4P1	27
31	M	25	B	G	NAG	B1C3E1P1	3
32	F	19	D	R	PAG	B1C1E1P1	1
33	M	17	B	R	PAG	B1C1E1P1	1
34	M	17	D	R	O6	B1C1E1P1	1
35	F	17	C	M	NAG	B5C1E3P1	28
36	M	13	C	M	PAG	B1C3E1P8	12
37	F	11	D	SR	NAG	B1C9E1P9	20
38	F	10	A	SR	NAG	B8C5E1P3	31
39	F	10	B	SR	NAG	B1C1E1P1	1
40	F	6	B	SR	PAG	B1C1E1P5	5
41[f]	M	14	C	M	NAG	B1C1E1P1	1
42[f]	M	14	C	G	NAG	B1C3E1P1	3
43**	M	15	B	SR	PAG	B1C1E1P1	1
44**	M	17	C	D	PAG	B1C2E1P1	2
45	M	13	A	SR	NAG	B1C4E1P1	6
46	F	14	C	S	NAG	B6C6E1P8	29
47	F	9	C	SR	O6	B1C3E1P1	3
48	M	18	D	S	O6	B1C1E1P1	1
49***	M	9	B	R	O6	B1C1E1P3	9
50	F	9	A	M	NAG	B1C1E1P1	1
51***	F	7	A	SR	NAG	B1C1E1P1	1
52	M	16	D	SR	NAG	B1C1E1P1	1
53	M	4	A	SR	PAG	B1C2E1P1	2
54	F	9	B	G	NAG	B1C1E1P5	5
55	F	14	C	G	O6	B1C1E1P1	1
56	F	15	B	R	O6	B1C3E1P1	3
57	M	17	C	M	NAG	B1C1E1P1	1
58	F	15	B	SR	PAG	B1C2E1P1	2
59	F	8	A	S	NAG	B1C1E1P1	1
60	F	16	C	SR	NAG	B1C1E1P1	1
61	F	18	D	D	O3	B1C1E1P1	1
62	F	16	B	SR	PAG	B1C1E2P3	10
63	M	15	B	M	NAG	B1C4E1P1	6
64	M	11	D	SR	NAG	B1C10E2P1	21
65	M	25	C	G	NAG	B1C1E1P2	4

Continued on following page

TABLE 1—*Continued*

Patient					Strain		
No.[a]	Sex[b]	Age (yr)	Clinical state[c]	Morphology[d]	Serotype[e]	Ribotype	Ribotype no.
66	M	20	D	SR	PAG	B9C2E1P1	32
67	F	12	C	SR	PAG	B1C1E1P5	5
68	M	14	A	G	NAG	B1C1E1P1	1
69	F	9	B	G	O3	B1C1E1P1	1
70	M	23	B	SR	NAG	B1C3E1P1	3
71	M	2	A	M	NAG	B1C1E1P1	1
72	M	19	C	S	PAG	B2C7E1P4	23
73	M	5	B	S	NAG	B2C7E6P4	24
74	M	5	B	D	O6	B1C1E5P1	11
75	F	15	A	M	NAG	B1C4E1P6	14
76	F	12	B	M	NAG	B1C1E1P1	1
77	F	8	D	D	NAG	B1C1E1P1	1
78	M	6	B	R	NAG	B1C1E1P1	1
79	F	14	B	R	PAG	B1C5E1P1	16
80	M	7	C	SR	PAG	B2C2E2P3	22
81	F	1	A	S	PAG	B1C2E1P1	2
82	F	3	A	D	PAG	B1C4E3P1	15
83	M	11	B	SR	PAG	B1C2E1P1	2
84	M	33	E	SR	NAG	B1C2E1P1	2
85	F	14	A	R	O13	B1C6E2P1	19
86	M	11	B	S	O6	B1C6E1P3	18
87	F	34	B	S	PAG	B10C2E2P1	33
Reference[g]				SR	O6	B1C2E2P4	34

[a] Patients with the same symbols (*, **, ***) are siblings.
[b] F, female; M, male.
[c] See Materials and Methods for details.
[d] S, smooth; R, rough; M, mucoid, G, gelatinous; D, dwarf; SR, smooth-rough.
[e] NAG, nonagglutinable; PAG, polyagglutinable.
[f] Twin brothers.
[g] ATCC 10145 reference strain.

Colony morphology. At initial isolation, clinical isolates of *P. aeruginosa* were each classified as one of the six following morphological types after growth on nutrient agar no. 1 (Unipath Ltd., Basingstoke, England) (28): smooth, rough (large), mucoid, gelatinous, dwarf, and smooth-rough (enterobacter).

Serotyping. Serotyping was performed by slide agglutination with commercial O antisera (Diagnostic Pasteur, Marnes La Coquette, France) according to the international antigenic typing sheme of Habs (12) with the subtypes of Véron (27).

Bacterial DNA preparation. Each strain was subcultured aerobically on two nutrient agar plates (15 by 15 cm) for 18 to 24 h at 37°C. The cultures were checked visually for purity, harvested in 15 ml of buffer (40 mM Tris, 2 mM EDTA [pH 8.0]), and lysed by adding 220 μl of a 25% (wt/vol) aqueous solution of sodium dodecyl sulfate (SDS) and 30 μl of pronase (Sigma, St. Louis, Mo.). The mixture was incubated overnight at 37°C to allow cell lysis. DNA was extracted and purified as described by Brenner et al. (2).

rDNA RFLP patterns. rDNA RFLP patterns were determined by a modification (16) of the method described by Grimont and Grimont (10). DNA (5 μg) was digested with *Bam*HI, *Cla*I, *Eco*RI, or *Pst*I restriction enzymes (REs; Boehringer, Mannheim, Germany) according to the manufacturer's instructions, and the fragments were separated by horizontal electrophoresis for 16 h at constant voltage (50 V) in a 0.8% agarose gel (Sigma type II) made in TBE buffer (130 mM Tris, 80 mM borate, 2.5 mM EDTA [pH 8.0]). The molecular weight standard Raoul I (Appligene, Illkirch, France), which contains 22 DNA fragments (234 to 48,502 bp), and DNA of the type strain (ATCC 10145) were included in each gel. Gels were stained with ethidium bromide (1 μg/ml) for 30 min, and the DNA fragments were visualized under UV light.

DNA fragments were vacuum transferred to a nylon membrane (GeneScreen Plus; NEN Research, Boston, Mass.) as recommended by the manufacturer (Vacuoblot; Pharmacia, Uppsala, Sweden).

Probes with a high specific activity (6×10^7 dpm) were prepared from 250 ng of *E. coli* 16S plus 23S rRNA (Boehringer) by random oligopriming with a mixture of hexanucleotides (Pharmacia), cloned Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, Md.), and [^{32}P]dCTP (800 Ci/mmol; Amersham, Buckinghamshire, England) according to the manufacturers' instructions.

The probes were hybridized to the membrane for 3 h at 65°C, and the membranes were then washed twice with 0.15 M NaCl and 0.015 M sodium citrate, containing 0.1% SDS, for 15 min at room temperature (16). The filters were autoradiographed by exposure to X-OMAT AR film (Eastman Kodak Company, Rochester, N.Y.).

Autoradiographs of each gel were digitized with a video camera connected to a microcomputer (Bio-Profil; Vilbert Lourmat, Marne-la-Vallée, France). The images in TIFF format were transferred to a Macintosh computer, and the lanes and bands were detected by using RestrictoScan software (Institut Pasteur, Paris, France). The migration data generated by the RestrictoScan software were recaptured by RestrictoTyper, a program (Institut Pasteur) which generates and prints a schematic representation of electrophoretic patterns. Each different combination of rDNA RFLP patterns obtained with the four REs was defined as a distinct ribotype.

Statistical analysis. The data were summarized by a two-way table of 88 rows (the strains) and a number of columns corresponding to the number of different rDNA fragments with a distinct molecular size and detected in any strain after *Bam*HI, *Cla*I, *Eco*RI, or *Pst*I digestion. The value of each cell is 1 (present) or 0 (absent) (15). From this table, a dissimilarity matrix was constructed and was used for hierarchical clustering by using a PW^2 Advantage Unisys Computer by means of STAT-ITCF (Institut Techniques des Céréales et des Fourrages, Paris) (3).

RESULTS

Clinical evaluation. The clinical conditions of the 87 patients are summarized in Table 1.

Colony morphology and serotyping. The distribution of colony morphologies was as follows: dwarf, 10 strains; gelatinous, 9 strains; mucoid, 10 strains; smooth, 13 strains; rough, 9 strains; and smooth-rough, 36 strains (Table 1). Seventeen of the clinical isolates were O serotypeable (20%). They belonged to serogroups 1 (1 strain), 3 (3 strains), 6 (12 strains), and 13 (1 strain). Forty strains (46%) and 30 strains (34%) were nonagglutinable and polyagglutinable, respectively (Table 1).

rRNA gene RFLPs. We used four REs (*Bam*HI, *Cla*I, *Eco*RI, and *Pst*I) that were previously used to demonstrate the

TABLE 2. Distribution of ribotypes among the 87 isolates of *P. aeruginosa* according to the clinical status of the patient

Ribotype	No. of patients with the following clinical status[a]:					Total
	A	B	C	D	E	
R1	5	8	10	6	0	29
R2	3	3	3	0	2	11
R3	0	4	3	2	0	9
R4	0	0	3	0	0	3
R5	0	2	1	0	0	3
R6	1	1	1	0	0	3
R7	0	1	1	0	0	2
R8	0	1	1	0	0	2
R9-33	4	8	7	6	0	25
Total	13	28	30	14	2	87

[a] See Materials and Methods for details.

extensive genetic heterogeneity of *P. aeruginosa* strains (1, 11). For the 87 isolates from patients with CF, 10 rDNA RFLPs were obtained with *Bam*HI (labeled B1 to B10), 10 were obtained with *Cla*I, (C1 to C10), 6 were obtained with *Eco*RI (E1 to E6), and 9 were obtained with *Pst*I (P1 to P9). The number of different identifiable rDNA fragments in the total population was 13 after *Bam*HI restriction, 10 after *Cla*I restriction, 8 after *Eco*RI restriction, and 11 after *Pst*I restriction. All of these rRNA gene RFLP patterns are represented schematically in Fig. 1, and a blot of the three most frequent patterns obtained with each RE is presented in Fig. 2. The RFLPs with the four enzymes identified 33 ribotypes, named R1 to R33, in the total population of clinical strains (Table 2). The ATCC 10145 reference strain was the only strain of ribotype R34. All strains were subcultured, and their DNAs were subsequently extracted and tested at least three times. The rDNA RFLP patterns were not altered after subculture.

Cluster analysis of rDNA RFLP data generated the dendrogram presented in Fig. 3. Seventy-one of the 88 strains (81%) clustered within a distance of less than one-third of the greatest distance observed in the total population, and the three major ribotypes (R1 to R3) were closely related (Fig. 3).

Nine strains were obtained from siblings with CF in five families. In one family, two of the three siblings (patients 17 and 18) were colonized with strains with the same ribotype, ribotype R4 (Table 1), whereas the third sibling was colonized with a strain belonging to ribotype R7, which can be distinguished from ribotype R4 only by the *Cla*I RE pattern (C3 and C1, respectively). In three other families, the differences between the ribotypes of strains from siblings were confined to the *Cla*I RE pattern (patients 41 and 42, 43 and 44, and 49 and 51). Only strains isolated from patients of the last family (patients 49 and 51) were very different (ribotypes 1 and 9; Fig. 3).

Relationships between colony morphology, serotype, rRNA gene RFLPs, and clinical stage. No relationships between colony morphology and serotype or between colony morphology and rRNA gene RFLPs were observed. Nevertheless, most of the strains of ribotype R1 were nonagglutinable (19 of 29 strains). By contrast, 8 of the 11 strains of ribotype R2 were polyagglutinable.

There was no correlation between ribotype and the clinical stage of the disease (Table 2).

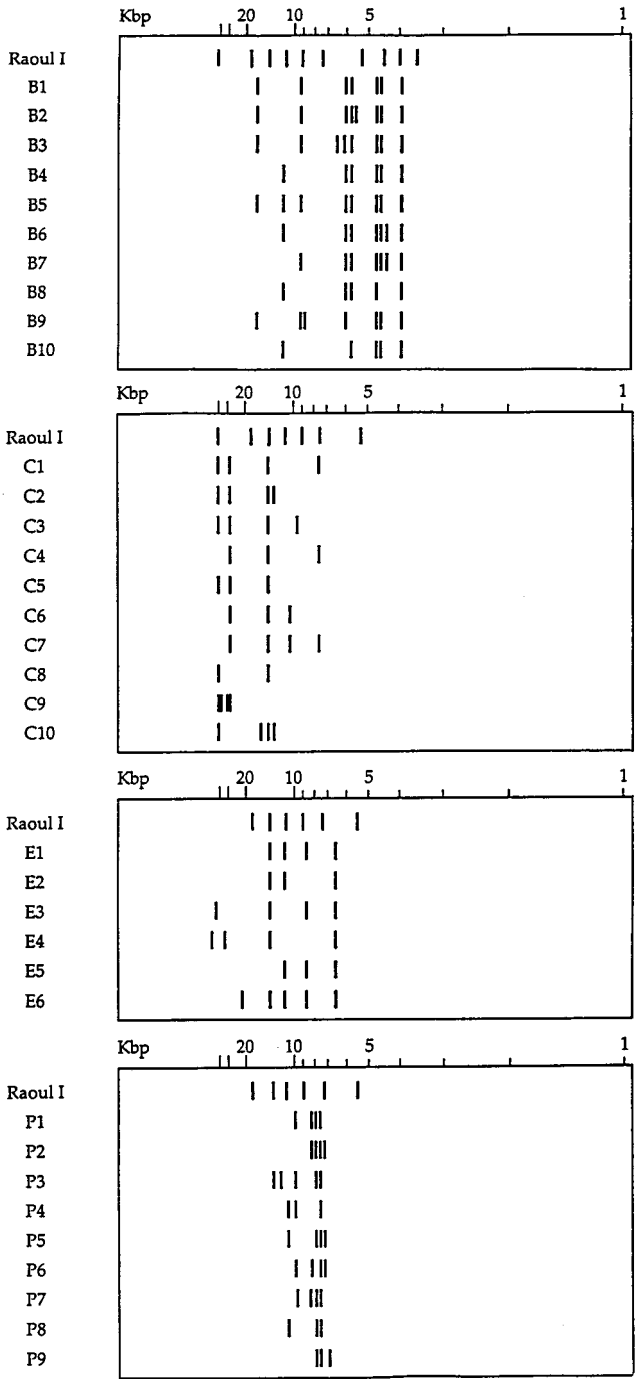

FIG. 1. Schematic representation of rDNA RFLP patterns obtained after *Bam*HI (B1 to B10), *Cla*I (C1 to C10), *Eco*RI (E1 to E6), and *Pst*I (P1 to P9) digestion of total DNA from 88 strains of *P. aeruginosa*. Lane Raoul I, DNA molecular mass marker which contained fragments of 48.5, 18.5, 15.0, 10.6, 9.0, 7.4, 5.6, 4.4, 4.0, and 3.6 kbp.

DISCUSSION

rDNA RFLP analysis or ribotyping is a stable and reproducible technique for determining the genetic diversity of many organisms (10). Indeed, this method has been used to study the polymorphisms of *E. coli* (16, 26), *Pseudomonas cepacia* (26), and *H. influenzae* (18, 26).

The ability of this method to distinguish between unrelated isolates of *P. aeruginosa* was demonstrated by Blanc et al. (1). They showed that ribotyping with *Bam*HI, *Cla*I, *Eco*RI, and *Pst*I gives a high index of discrimination and therefore validates

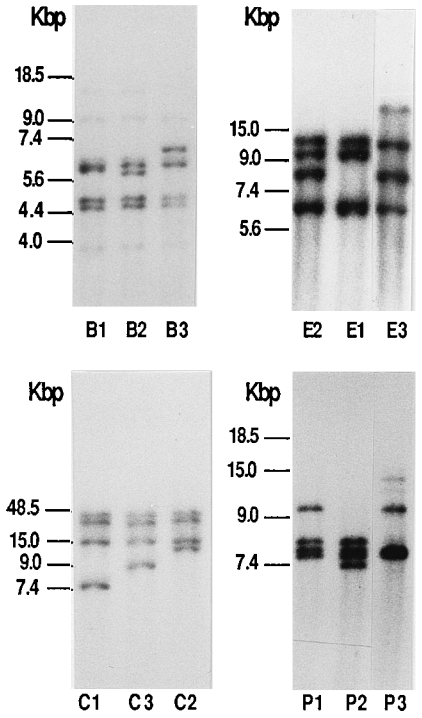

FIG. 2. Examples of most frequent *Bam*HI, *Cla*I, *Eco*RI, and *Pst*I rDNA RFLP patterns. For details, see the legend to Fig. 1.

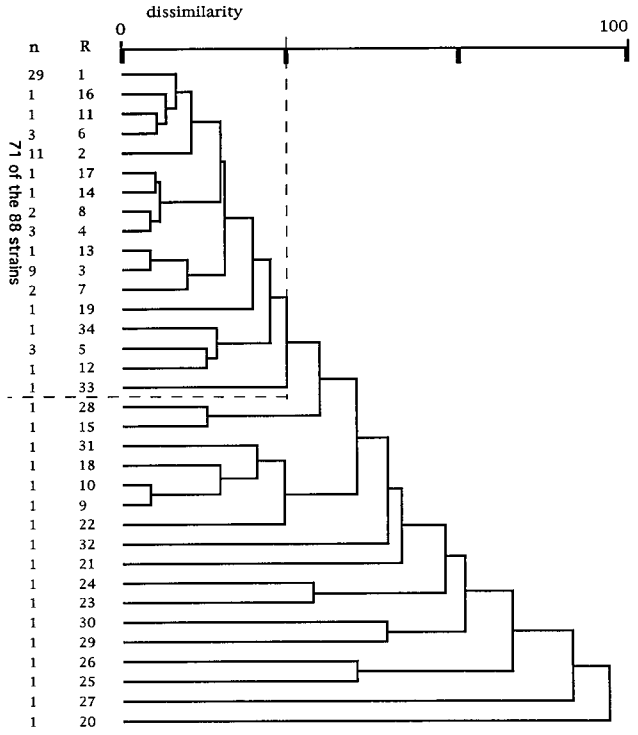

FIG. 3. Dendrogram prepared from the distances obtained from the dissimilarity matrix on the basis of the rDNA RFLP data after restriction by *Bam*HI, *Cla*I, *Eco*RI, and *Pst*I. The distance used was the distance of Chi-2. The ribotype (R) and the number of strains (n) belonging to each ribotype are indicated. Seventy-one of the 88 *P. aeruginosa* strains (81%) from patients with CF clustered within a distance of less than one-third of the greatest distance observed in the total population.

the method for discriminating between clinical *P. aeruginosa* isolates. Using this tool, they showed that rRNA genes of various clinical isolates of *P. aeruginosa* are diverse. Since then the heterogeneity of strains of *P. aeruginosa* isolated from patients with various pathologies has been confirmed by many studies (8, 11). The extensive genetic heterogeneity of *P. aeruginosa* clinical isolates is also suggested by the absence of a correlation between serotyping, esterase typing, and ribotyping results (4, 14).

The genetic diversity of strains isolated from patients with CF has been less clear and the subject of prolonged debate. Results of studies that have used Southern hybridization with different DNA probes have shown the genomic homogeneity of *P. aeruginosa* strains isolated from the airways of patients with CF (6, 24). Those studies indicate that more than 40% of patients harbor the same type of strains. However, most of the specimens were obtained from patients who were attending the same hospital. The observed homogeneity of strains may thus have resulted from cross colonization.

Macrorestriction analysis of total DNA of *P. aeruginosa* strains from patients with CF has given conflicting results. Predominant clones were observed in each of four clinics studied by Römling et al. (19). In other studies, the results of genome fingerprinting of *P. aeruginosa* isolates from patients with CF argued for a greater heterogeneity (25). Nevertheless, the degree of relatedness of strains exibiting different profiles after macrorestriction of total DNA is difficult to establish.

We examined rRNA genes after restriction by four endonucleases which were highly discriminant, with the exception of serotype O12, when they were used to study *P. aeruginosa* strains from patients without CF (1, 11). The objective was to estimate the diversity of these genes in a population of *P. aeruginosa* strains collected from patients with CF living in diverse regions of France. Fifty-six percent of the strains clustered in the three most frequent ribotypes. In addition, computer analysis of the data in terms of population structure showed that 81% of the strains were closely related (Fig. 3). These results suggest a homogeneity of rRNA genes in *P. aeruginosa* strains from patients with CF, a homogeneity which was observed independently of the clinical status of the patient and the colony morphology.

A comparison of our data with those of Blanc et al. (1) (the two studies used the same four REs) shows that the genetic diversity of the rRNA genes of isolates of *P. aeruginosa* from French patients with CF is lower than that of the rRNA genes of strains isolated from various specimens including blood culture, sputum, urine, and ear swab specimens from patients without CF or from the environment. Among these unrelated isolates, Blanc et al. (1) found 33 ribotypes among 55 *P. aeruginosa* strains. In addition, their two most frequent ribotypes were composed of 15 strains only (27% of the strains), while our two most frequent ribotypes included 40 of the 87 strains (46% of the strains) from patients with CF. When, like Blanc et al. (1), we did not include the very weak bands in patterns obtained with *Bam*HI, the two most frequent ribotypes, ribotypes R1 and R2, in the two studies were similar. This suggested that the two groups of *P. aeruginosa* strains frequently colonizing the airways of patients with CF could also be associated with other diseases (urinary tract, airway, and wound infections and pancreatic abscesses) or could be isolated from the environment.

The difference between results of rDNA RFLP analysis and genome fingerprinting can be explained. The conserved rDNA regions of *P. aeruginosa* strains isolated from unrelated pa-

tients with CF are consistent with the idea that a limited number of clones colonize the airways of patients with CF, but recombination or horizontal genetic transfer during the long evolution of the disease may cause divergence of other genes resulting in different pulsed-field electrophoresis patterns. The origins of these predominant clones may be strains belonging to the aquatic environment, as suggested by recent work (19). Nevertheless, all of their patients with CF were French and may have been in contact in treatment centers or nursing homes. Therefore, cross colonization may have increased the diffusion of particular groups of *P. aeruginosa* strains.

The low degree of heterogeneity of rRNA operons of *P. aeruginosa* strains from patients with CF indicates that there is little hope that ribotyping could be a valuable method for epidemiological studies in patients with CF when ribotyping is used alone. Combination of several molecular typing methods would improve the degree of discrimination (4). Nevertheless, the poor understanding of the *P. aeruginosa* population structure makes comparison of two apparently different strains and particular assessments of their relatedness difficult.

Studies of several parts of the genome of *P. aeruginosa* strains isolated from patients with CF, patients without CF, and environmental sources in terms of population structure by computation would help to clarify the genetic complexity of *P. aeruginosa* strains from patients with CF.

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