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Random Amplified Polymorphic DNA Typing of *Pseudomonas aeruginosa* Isolates Recovered from Patients with Cystic Fibrosis

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***Pseudomonas aeruginosa* isolates recovered from chronically colonized patients with cystic fibrosis (CF) are phenotypically different from those collected from other patients or from the environment. To assess whether alterations in motility, mucoidy, and serum susceptibility represented an adaptation to chronic infection or replacement by a new strain, sequential *P. aeruginosa* isolates of known phenotype collected from 20 CF patients were typed by random amplified polymorphic DNA (RAPD) analysis. A total of 35 RAPD strain types were found among 385 isolates from 20 patients, and only two patients had *P. aeruginosa* strains of the same RAPD fingerprint. Eight strain pairs representative of the first eight RAPD types were also analyzed by *Spe*I macrorestriction followed by pulsed-field gel electrophoresis (PFGE); the strain types found by both fingerprinting techniques correlated exactly. In 11 of 20 patients, the RAPD types of serial *P. aeruginosa* isolates remained stable despite alterations in isolate motility, colonial morphology, and lipopolysaccharide phenotype. However, in isolates collected from one CF patient, a single band change in RAPD fingerprint and *Ceu*I PFGE profile correlated with the appearance of an RpoN mutant phenotype, suggesting that the altered phenotype may have been due to a stable genomic rearrangement. Secretion of mucoid exopolysaccharide, loss of expression of RpoN-dependent surface factors, and acquisition of a serum-susceptible phenotype in *P. aeruginosa* appear to evolve during chronic colonization in CF patients from specific adaptation to infection rather than from acquisition of new bacterial strains.**

Pseudomonas aeruginosa is the leading cause of lung infection and death in patients with cystic fibrosis (CF). Several *P. aeruginosa* virulence factors which may contribute to pathogenesis during (i) initial colonization of the respiratory tract and (ii) persistence during chronic infection have been characterized. Pili, nonpilus adhesins, and bacterial motility have been shown to be important in initial colonization of the respiratory tract (14, 15, 17, 25), and phenotypic characterization of primary *P. aeruginosa* isolates from CF infection has shown them to be highly motile and express pilin and flagellin (11). During the subsequent chronic colonization of the respiratory tracts of CF patients, alteration in secretion of mucoid exopolysaccharide, exotoxins, proteases, and siderophores; conversion of lipopolysaccharide from smooth to rough; and a change to nonmotility may play a significant role in the ability of *P. aeruginosa* to evade host defenses and successfully maintain infection (4, 5, 11, 12, 21).

The peculiar phenotype of *P. aeruginosa* isolates recovered from chronic CF infection is generally believed to result from adaptation of the colonizing strain, but the relationship between strain genotype and phenotype has not been examined in depth. Genetic typing techniques have been shown to be more discriminatory than phenotypic methods for typing *P. aeruginosa* CF isolates (7). Restriction fragment length polymorphism (RFLP) analysis of the *toxA* and *pilA* loci demonstrated that most CF patients are chronically colonized with isolates of a single RFLP type for prolonged periods of time (13, 23); coinfection and strain replacement have also been reported in CF infection when strains were typed by single-locus probe typing (2, 29). Macrorestriction of *P. aeruginosa* CF isolates and examination of genomic fingerprints by pulsed-field gel electrophoresis (PFGE) have also demonstrated the persistence of single clones in some CF patients (3, 16, 24) as well as coinfection with one or more strains (24). DNA fingerprinting of *P. aeruginosa* CF isolates by arbitrary-primed or random amplified polymorphic DNA (RAPD) PCR (27, 28) has also recently been reported (6, 8). This rapid, PCR-based methodology has been shown to be as discriminatory as PFGE for typing *P. aeruginosa* and was recommended for the primary screening of large numbers of isolates because of its efficiency (8).

Although previous genetic typing studies have independently correlated loss of piliation (23), alterations in mucoidy (2), changes in antimicrobial susceptibility (24), and loss of pyocin and phage typing responses (16) with strain type, our study is the first to systematically examine the strain type during phenotypic alterations in motility, mucoidy, and serum susceptibility of sequential CF isolates. In a previous study, we examined alterations in motility and mucoidy of more than one thousand sequential *P. aeruginosa* CF isolates recovered from 20 patients (11). Using a RAPD method which is unique in that it utilizes arbitrary 10-base primers identified specifically for their ability to discriminate *P. aeruginosa*, we have analyzed the

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TABLE 1. Sequences of 10-mer RAPD primers producing reproducible polymorphisms with *P. aeruginosa*

Primer	Sequence (5′ to 3′)
208	ACGGCCGACC
228	GCTGGGCCGA
241	GCCCGAGCGG
270	TGCGCGCGGG
272	AGCGGGCCAA
275	CCGGGCAAGC
277	AGGAAGGTGC
287	CGAACGGCGG

genotypes of *P. aeruginosa* CF isolates (385 in total) from the latter phenotypically characterized collection (11). RAPD fingerprint analysis of sequential *P. aeruginosa* CF isolates which have undergone phenotypic alterations in motility, mucoidy, and serum susceptibility forms the basis of this report.

MATERIALS AND METHODS

Bacterial strains and culture. *P. aeruginosa* isolates were obtained from 20 patients attending the Cystic Fibrosis Assessment Clinic at British Columbia's Children's Hospital and the Adult Cystic Fibrosis Clinic at St. Paul's Hospital, Vancouver, British Columbia, Canada. Isolation, culture, and determination of *P. aeruginosa* motility phenotype (expression of flagellin and ability to spread in soft agar) have been described previously (11). A minimum of 9 isolates spanning the period of colonization studied for each patient were analyzed by a RAPD method, and a total of 385 CF isolates were typed. International Antigenic Typing Scheme (IATS) strains of *P. aeruginosa* were provided by Robert Hancock, University of British Columbia.

Isolation of *P. aeruginosa* genomic DNA. A single colony was inoculated into 2 ml of L broth (in a 13-ml snap-top tube) and grown overnight with end-over-end rotation at 37°C. After harvest by centrifugation (3,000 × *g*, 10 min), the bacterial pellet was resuspended in 0.4 ml of GET buffer (50 mM glucose, 70 mM EDTA, 50 mM Tris-Cl [pH 8]), and 0.2 ml was transferred to a 1.5-ml screw-cap microcentrifuge tube containing approximately 0.5 ml of 0.1-mm-diameter glass beads and 0.9 ml of lysis buffer (50 mM Tris-Cl [pH 8], 50 mM EDTA, 1% sodium dodecyl sulfate, 30 μg of RNase per ml). The bacteria were then lysed by a 3-min pulse on a mini-bead-beater device (Biospec Products, Bartlesville, Okla.), and RNA was digested by incubation of the lysate at 37°C for 1 h. After incubation, the lysate was cleared by brief centrifugation and 0.7 ml was removed to a fresh tube. One-third volume of saturated ammonium acetate was added, and the contents of the tube were mixed vigorously; the resulting protein and polysaccharide precipitate was removed by centrifugation, and genomic DNA was collected from the cleared lysate by ethanol precipitation. After the DNA pellet was washed with 70% ethanol, it was dissolved in 100 μl of TE (Tris-EDTA) (pH 8) (18), and the DNA was quantitated by A_{260}.

RAPD analysis. One hundred RAPD primers (10-mers) of arbitrary sequence were obtained from John Hobbs, Nucleic Acid and Protein Service Unit, University of British Columbia, Vancouver, British Columbia, Canada. All 100 primers were initially screened for the ability to produce discriminatory polymorphisms by using 20 ng of control DNA isolated from *P. aeruginosa* PAK, PAOI, and P1 in the PCR mixtures described below. Eight primers were found to amplify reproducible DNA fingerprints (see Table 1); primer 272 was primarily used to type the CF isolates described in this study, and primer 208 was used for confirmation.

RAPD PCR mixtures were set up and incubated exactly as previously described (1). Reactions mixtures (25 μl) were made optimum for *P. aeruginosa* and contained 40 ng of genomic DNA, 40 pmol of oligonucleotide, 1 U of *Taq* polymerase (GIBCO-BRL, Gaithersburg, Md.), 250 μM (each) deoxynucleoside triphosphate (Ultra-Pure, Pharmacia, Laval, Quebec, Canada), 10 mM Tris-Cl (pH 8), 50 mM KCl, 0.001% gelatin, and 3 mM $MgCl_2$. Each reaction mixture was overlaid with 25 μl of mineral oil and amplified with a Perkin-Elmer Cetus DNA Thermal Cycler model TC-1 as follows: (i) 4 cycles, with 1 cycle consisting of 5 min at 94°C, 5 min at 36°C, and 5 min at 72°C and (ii) 30 cycles, with 1 cycle consisting of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C, followed by a final extension step at 72°C for 10 min.

RAPD products (one-third of each reaction mixture) were then separated by electrophoresis in 1.5% agarose gels (20 wells; 11 by 14 cm) with 1× TBE running buffer (18) at 9 V/cm for 3 h. Molecular size standards were included on all gels (1-kb ladder; GIBCO-BRL). After the gels were stained with ethidium bromide, they were photographed onto film (4 by 5 in. [10.16 by 12.7 cm]) (TRI-X pan; Eastman Kodak Co., Rochester, N.Y.). The RAPD fingerprints were analyzed both by eye and also by computer with GelManager for Windows V1.5 software (Biosytematica, Prague, Czech Republic), with the molecular size standards used to correct for gel-to-gel migration variation. Similarity coefficients of the RAPD fingerprints were calculated from the entire absorbance profiles with the Identify program of GelManager for Windows.

PFGE. Genomic fingerprinting of *P. aeruginosa* isolates by PFGE was performed exactly as described previously (9). Endonuclease-digested DNAs were separated by electrophoresis in 1.4% agarose gels (0.5× TBE running buffer) (18) with a CHEF-DR2 PFGE device (Bio-Rad Laboratories, Mississauga, Ontario, Canada) at 16°C under the following ramp conditions: 5 to 40 s for 14 h and 1 to 100 s for 29 h at a field strength of 11 V/cm. Lambda DNA concatemers (New England Biolabs, Mississauga, Ontario, Canada) were included as size standards. PFGE fingerprints were compared by eye.

Serum susceptibility. The susceptibilities of sequential *P. aeruginosa* CF isolates to the bactericidal effect of human serum were determined as previously described (22). The susceptibility of each strain was assessed on three separate occasions, and the mean ± standard error of the mean (in CFU per milliliter) was recorded. Control strains of *P. aeruginosa* P1 (serum sensitive) and M2 (serum resistant) were included in all experiments, and a serum-sensitive CF isolate was defined as one in which ≥90% of the initial inoculum was killed after 2 h of incubation (22).

RESULTS

Identification of primers for RAPD analysis of *P. aeruginosa*. Eight primers were found to amplify reproducible polymorphisms suitable for strain differentiation of *P. aeruginosa*. The sequence of each primer is shown in Table 1, and the polymorphisms amplified from DNAs extracted from two subcultures of *P. aeruginosa* PAK made from the same freezer vial on two separate occasions are shown in Fig. 1A. The functional primers (Table 1) were all of high GC content (60 to 90%) compared with those which did not amplify RAPD products

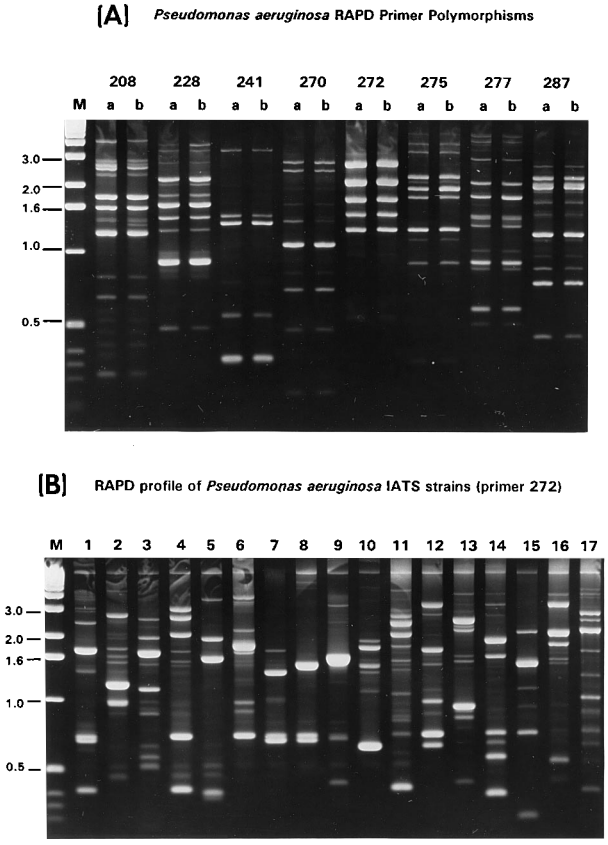

FIG. 1. *P. aeruginosa* strain-specific polymorphisms generated by different RAPD primers. The polymorphisms were amplified by each primer (sequences given in Table 1) from *P. aeruginosa* PAK. DNA was prepared on two separate occasions (indicated by a and b over the lanes) from the same frozen bacterial stock (A). The polymorphisms generated from each of the 17 IATS strains by primer 272 are shown in panel B. Molecular size markers were run in lanes M, and their sizes (in kilobases) are indicated to the left of the gels.

from *P. aeruginosa* DNA (40 to 60%). Each of the primers amplified polymorphisms which ranged from 5 to 20 bands over a size range of 100 bp to 5 kb and which were reproducible for the two independent preparations of strain PAK DNA (Fig. 1A). The RAPD polymorphisms remained stable with 10 to 100 ng of template DNA and 10 to 160 pmol of primer present in the reaction mixtures. A minimum of 5 ng of template DNA was required to produce a complete fingerprint (data not shown). Each primer yielded RAPD patterns which allowed unique strains of *P. aeruginosa* to be differentiated; the polymorphisms generated from the *P. aeruginosa* IATS strains with primer 272 are shown in Fig. 1B (data not shown for other primers). The highest similarity coefficient of the RAPD patterns generated by primer 272 from the IATS strains was 0.70 (between IATS strain 7 and 8). The remaining useful primers amplified RAPD profiles from the IATS strains, which although strain specific, demonstrated a greater degree of fingerprint similarity than primer 272 (data not shown). Primer 272 was used to type the CF isolates described in this study, and primer 208 was used for confirmation; none of the other primers described in Table 1 was evaluated further.

RAPD fingerprinting of sequential *P. aeruginosa* CF isolates. A total of 385 *P. aeruginosa* CF isolates were typed by RAPD analysis with primer 272 to determine the stability of the strain genotype during phenotypic conversion. The RAPD typing results are summarized in Table 2. A minimum of nine isolates per patient spanning the longest period of collection available were selected. All available *P. aeruginosa* isolates recovered from patients 2, 3, 4, 12, and 15 were typed. RAPD fingerprints that possessed similarity coefficients of greater than 0.8 when examined side by side were considered identical and assigned a RAPD type. Thirty-five unique RAPD types and one subtype were found (Table 2). Patients 1, 5, 8, 9, 10, 11, 12, 13, 16, and 17 were chronically colonized with strains of a single RAPD type. Patients 5 and 10 were both colonized with a strain of RAPD type 7. Five patients (patients 2, 6, 7, 14, and 18) were cocolonized at one time or another with two or more strains of different RAPD type. Patients 3, 15, 19, and 20 demonstrated replacement of one *P. aeruginosa* RAPD type with another; however, there was a gap in recovery of strains between identification of different types (Table 2).

The RAPD fingerprint profiles obtained with primer 272 of the first 17 CF RAPD types are shown in Fig. 2. The fingerprints were highly conserved for sequential isolates of the same type (data shown for a pair of strains from each patient in Fig. 2, except for patient 4 [see below]) and remained stable for isolates collected over several years. For example, isolates 1913 and 4503 (type 1; Fig. 2) were recovered 5.2 years apart from patient 12, isolates 1823 and 5471 (type 7; Fig. 2) were collected 7.1 years apart from patient 10, and isolates 971 and 4566 (type 11; Fig. 2) were recovered 7.8 years apart from patient 13. The RAPD fingerprints obtained with primer 272 were discriminatory among CF strain types, with different numbers, sizes, and intensities of amplified DNA bands. Each RAPD type designated with primer 272 was confirmed with primer 208, although these fingerprints possessed more bands which were conserved between types and fewer bands which were discriminatory between types (data not shown).

Correlation of RAPD fingerprints with PFGE macrorestriction profile. A total of 16 CF isolates (eight isolate pairs representative of the first 8 RAPD fingerprint types) were also analyzed by PFGE after digestion with the enzyme *Spe*I. Two isolates (representative of RAPD types 5 and 6) were not typed by PFGE because of consistent nuclease contamination. The PFGE fingerprints of RAPD types 1, 2, 3, 7, 8, and 9 are shown in Fig. 3. Each pair of isolates yielded a unique and conserved macrorestriction fingerprint. The *Spe*I fingerprints were stable for each pair of sequential isolates examined, although single band changes were present in some of the isolate pairs (for example, isolates 1913 and 4503 and isolates 1823 and 5471; Fig. 3). For the limited number of *P. aeruginosa* CF isolates examined, the strain type found by both RAPD analysis and PFGE correlated exactly.

TABLE 2. RAPD types of sequential *P. aeruginosa* isolates from CF patients

Patient (sex)[a]	RAPD type of isolate	No. of isolates	Patient age (yr) when isolates were collected	Motility phenotype[b]
1 (F)	10	10	0.6[c]–3.8[d]	+
2 (F)	16	10	10–12.3	+
	17	38	10–19.4[d]	+
3 (F)	8	4	7.4–8.48	+
	9	38	11.6–14.6[d]	− to +
4 (M)	4	8	21.9–23.3	+
	4a	29	24.6–33.7	−
5 (M)	7	12	16.8–27.4	−
6 (F)	34	1	9.9	+
	35	1	10.1	−
	19	2	10.1, 12.0	−
	36	1	14.2	−
	33	3	12.0, 14.2, 16.9	+
	18	5	16.5–20.0[d]	+
7 (M)	20	1	22.3	+
	21	1	26.9	+
	15	7	28.0–32.1	+/−
	22	1	32.4	−
8 (M)	5	16	11.3–15.2[d]	+
9 (F)	3	9	4.3–14.8	+/−
10 (M)	7	16	10.8–17.9	−
11 (F)	32	20	22.8–29.1	+ to −
12 (M)	1	41	7.9[c]–15.1	+ to −
13 (M)	11	12	0.5[c]–8.31	+ to −
14 (M)	24	4	13.9, 15.7, 15.8	−
	25	2	15.3, 15.6	+
	26	4	15.7–22.4[d]	+ to −
15 (M)	6	7	5.1–6.0	+/−
	2	32	9.7–15.2	+ to −
16 (F)	13	10	4.5[c]–10.3	+
17 (F)	14	10	2.9[c]–8.7	+ to −
18 (F)	27	8	20.2–26.9[d]	+
	28	2	25.3, 25.9	−
19 (F)	29	1	3.1[c]	+
	12	9	6.8–11.5	+
20 (F)	30	1	3.5	+
	31	9	9.0–14.1	−
Total	35[e]	385		

[a] F, female; M, male.
[b] Motility phenotype adapted from data presented by Mahenthiralingam et al. (11). Symbols: +, motile isolates; −, nonmotile isolates; +/−, mixture of motile and nonmotile isolates.
[c] Age at colonization.
[d] Age at death.
[e] Number of RAPD types not including subtype 4a.

Stability of RAPD fingerprint during phenotypic alterations of *P. aeruginosa* isolates. Changes in isolate motility are summarized in Table 2. Patients 7, 9, 11, 12, 13, 14, 15, and 17 each yielded serial isolates of a distinct RAPD type which underwent loss of motility, indicating stable strain genotype during this phenotypic conversion (patient 14 was also colonized with two other isolate types; Table 2). Type 9 isolates recovered from patient 3 reverted back to a motile phenotype prior to death of the patient. Of the remaining sequential CF isolates

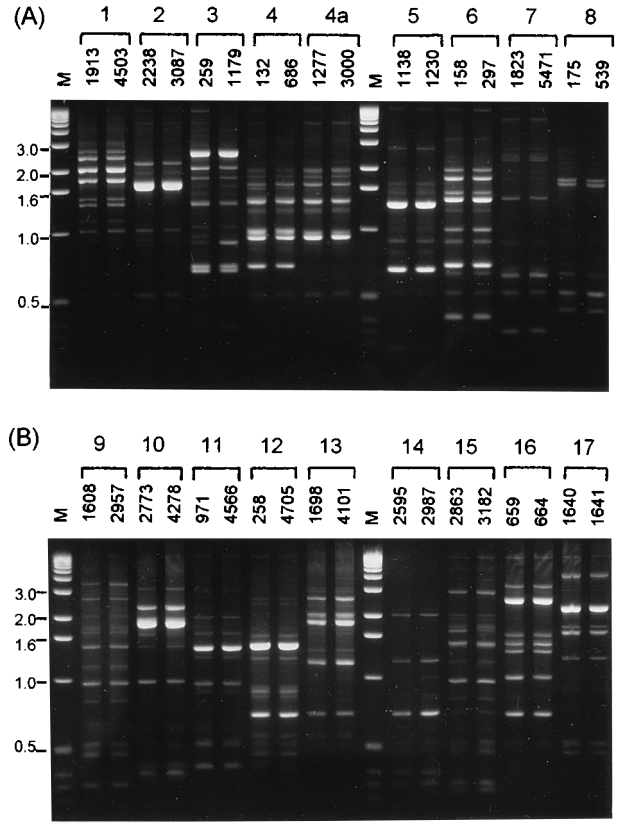

FIG. 2. *P. aeruginosa* CF isolate RAPD polymorphisms amplified by primer 272. The fingerprint patterns of 34 strains of 17 RAPD types are shown; type 1 to 8 (A) and types 9 to 17 (B) are shown. The strain number and RAPD type are indicated above each lane. Molecular size markers were run in lanes M, and their sizes (in kilobases) are indicated to the left of the gels.

examined, those of distinct RAPD type were either motile or nonmotile and showed no alteration in phenotype during the period studied. Type 7 isolates, which colonized patients 5 and 10, were also identical in motility phenotype, being piliated but not flagellated (11).

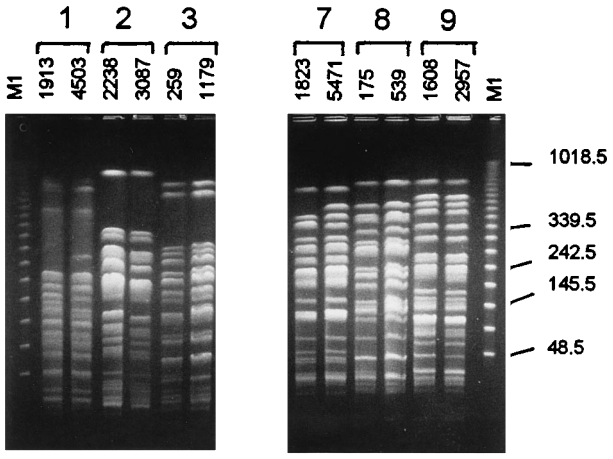

FIG. 3. *Spe*I PFGE fingerprints of six isolate pairs of unique RAPD types. The strain number and PFGE type are indicated above each lane. Molecular size markers were run in lanes M1, and their sizes (in kilobases) are indicated to the right of the gel.

TABLE 3. Phenotypic alterations of sequential *P. aeruginosa* isolates recovered from CF patients 1, 12, 13, 16, and 17

Patient and age (yr) at collection	RAPD type	Mucoidy[a]	Motility[b]	Serum susceptibility[c]
Patient 1				
0.60	10	−	+	R
1.60	10	−	+	R
2.35	10	−	+	R
3.18	10	+	+	S
3.35	10	−	−	S
3.76	10	+	+	S
Patient 12				
7.90	1	−	+	S
9.64	1	−	+	S
11.39	1	+	+	S
13.13	1	−	+	S
13.13	1	−	−	S
13.55	1	+	−	S
Patient 13				
0.50	11	−	+	R
4.32	11	−	+	R
4.32	11	+	+	R
7.89	11	−	−	S
7.89	11	+	−	S
8.31	11	−	−	S
Patient 16				
4.50	13	−	+	R
5.08	13	−	+	R
5.50	13	−	+	S
6.49	13	+	+	S
8.31	13	+	+	S
9.14	13	−	−	S
Patient 17				
2.90	14	−	+	R
3.72	14	−	−	R
5.05	14	−	−	S
7.71	14	+	−	S
8.13	14	+	−	S
8.70	14	−	−	S
Patient 19				
3.10	29	−	+	R
6.85	12	−	+	R
8.43	12	−	+	S
9.35	12	+	+	R
9.93	12	−	+	S
9.93	12	−	+	S
11.43	12	+	+	R

[a] Adapted from data presented by Mahenthiralingam et al. (11). Symbols: −, not mucoid; +, mucoid.

[b] Adapted from data presented by Mahenthiralingam et al. (11). Symbols: +, motile; −, not motile.

[c] Serum resistant (R) and sensitive (S), as defined by the criteria described in Materials and Methods.

Phenotypic alterations in expression of mucoidy, motility, and serum susceptibility of 6 sequential isolates recovered from patients 1, 12, 13, 16, 17, and 19 are shown in Table 3. The first isolate described for each patient represents the first time *P. aeruginosa* was cultured from his or her sputum and was considered the colonizing isolate (11). Five of these patients were each colonized with a distinct strain of *P. aeruginosa* (as indicated by RAPD analysis; Table 3) which persisted during the period studied. The primary *P. aeruginosa* isolate from patient 19 was type 29; this strain type was not subsequently

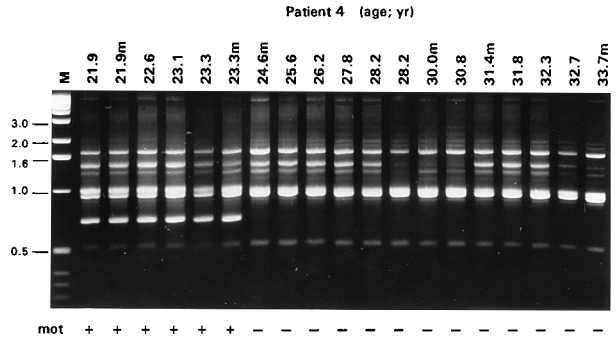

FIG. 4. RAPD analysis with primer 272 of sequential *P. aeruginosa* isolates collected from patient 4 in which conversion to a nonmotile phenotype occurred during chronic colonization. The patient's age at collection is indicated above each lane (m indicates that the isolate was mucoid), and isolate motility (mot) in soft agar is shown below each lane (motile [+]; nonmotile [−]). Molecular size markers are included in lane M, and their sizes (in kilobases) are indicated to the left of the gel.

recovered, and the patient became chronically colonized with type 12 isolates. All the colonizing isolates from the six patients were initially (i) nonmucoid (with mucoid strains of the same RAPD type appearing during chronic colonization) and (ii) motile (with a transition to a nonmotile but stable RAPD type, seen in patients 12, 13, and 17) and serum resistant (except for the primary isolate from patient 12). Serum-sensitive isolates of each patient's distinct RAPD type appeared during chronic colonization in all six patients; the primary isolate from patient 12 was serum sensitive from the first culture and remained that way during the period studied. Despite the phenotypic alterations observed in these serial isolates, the RAPD fingerprint remained stable (Table 3).

Genomic rearrangement associated with conversion of *P. aeruginosa* motility phenotype. RAPD analysis of the sequential isolates recovered from patient 4 demonstrated a correlation between phenotype and genotype. *P. aeruginosa* isolates were collected from patient 4 while the patient was 21.9 to 33.7 years old; the age at which this patient was first colonized with *P. aeruginosa* was not determined (11). Isolates collected over the first year of study were motile; however, all further isolates were nonmotile and possessed a phenotype similar to that of a *P. aeruginosa rpoN* mutant (11, 26) (nonflagellated, nonpiliated, and glutamine auxotrophic). A total of 37 isolates, 8 motile and 29 nonmotile, were evaluated by RAPD analysis; the polymorphisms generated by primer 272 from 19 isolates recovered from patient 4 are shown in Fig. 4. In contrast to the stable RAPD fingerprints of other sequential CF isolate types, there were distinct changes in the RAPD fingerprints (generated by primer 272) of the sequential isolates which correlated with isolate motility phenotype; a 0.7-kb band was lost and a doublet of bands at 1.0 kb was slightly altered in the RAPD fingerprints of nonmotile isolates from patient 4 (Fig. 4). The remainder of the RAPD fingerprint appeared conserved. Hence, the motile isolates were designated type 4 and the nonmotile, "RpoN mutant"-like isolates were designated type 4a.

Two type 4 (motile) and two 4a (nonmotile) isolates spanning the period of conversion in motility phenotype were examined by PFGE. The *Spe*I macrorestriction profile was conserved for all four isolates (Fig. 5A) unlike the RAPD fingerprints obtained with primer 272. When macrorestriction was performed with the enzyme *Ceu*I, which cuts only in the 23S rRNA gene (10), the macrorestriction profile was conserved for each pair of isolate types, 4 and 4a (Fig. 5B). The nonmotile isolates of type 4a demonstrated a reduction in the size of the smallest band cleaved by *Ceu*I. The conservation of the *Spe*I macrorestriction profile suggests that the strains colonizing patient 4 were clonal and derived from the same parental isolate. The stable difference in the *Ceu*I macrorestriction pattern, which directly correlated with the stable differences in RAPD fingerprint (Fig. 4), indicate that the parental strain may have undergone a genomic rearrangement. The stable genomic rearrangement coincided with the loss of strain motility and appearance of the "RpoN mutant" phenotype.

DISCUSSION

P. aeruginosa isolated from chronically colonized patients with CF possess a phenotype which is significantly distinct from strains collected from other patients or from the environment (4, 5, 11). In this report, we have systematically examined the relationship between the genotype of the infecting strain and the subsequent phenotypic conversion observed during chronic infection. Using an arbitrary-primed PCR typing method with primers screened for the ability to amplify discriminatory polymorphisms from *P. aeruginosa* DNAs, we examined the RAPD fingerprints of 365 sequential isolates recovered from 20 CF patients. In general, despite alterations in the expression of mucoid exopolysaccharide, bacterial motility, and acquisition of a serum-sensitive phenotype, the RAPD fingerprints of sequential isolates remained stable, suggesting that these changes result from phenotypic adaption of the primary colonizing isolates. However, sequential isolates recovered from one CF patient, which underwent a major change in motility resulting in a phenotype similar to that of an RpoN mutant (11), also demonstrated a concurrent change in RAPD profile and *Ceu*I PFGE profile indicative of genomic rearrangement. This represents the first correlation of genomic rearrangement with phenotypic alteration reported for isolates recovered directly from a chronically colonized CF patient.

RAPD has been utilized to type a wide range of bacteria including *P. aeruginosa* (6, 8) and the method described herein proved very useful for discrimination among *P. aeruginosa* CF isolates. Kersulyte et al. (8) recently described an arbitrary-

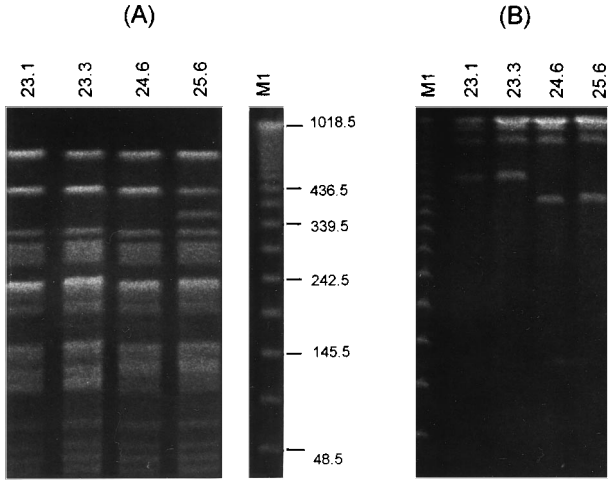

FIG. 5. PFGE fingerprints of isolates recovered from patient 4 before and after loss of isolate motility. The *Spe*I fingerprints of motile isolates collected when patient 4 was 23.1 and 23.3 years old, and nonmotile isolates collected when patient 4 was 24.6 and 25.6 years old (A) and the fingerprint patterns of the same isolates obtained after digestion with *Ceu*I (B) are shown. Molecular size markers were run in lanes M1, and their sizes (in kilobases) are indicated to the right of lane M1 in panel A.

primed PCR method which was able to distinguish *P. aeruginosa* CF strains with the same discriminatory power as macrorestriction (PFGE). Our method utilized 10-base primers which were selected from a random pool of 100 for suitability to type *P. aeruginosa*; Kersulyte et al. (8) found that RAPD analysis with 10-nucleotide primers lacked reproducibility and did not recommend these primers for typing *P. aeruginosa*. However, the 10-base primers tested in their study had previously been used for other microbial species (8) and were not screened specifically for the ability to generate strain-specific polymorphisms from *P. aeruginosa*. Our RAPD typing data suggest that 10-nucleotide primers can be used successfully for typing *P. aeruginosa* if they are identified specifically for that purpose. Typing by RAPD analysis also facilitated the analysis of many strains; the DNA extraction procedure required no phenol or chloroform extraction and its "minipreparation" scaling was well suited for large numbers of samples. All 385 CF isolates described in this study were typeable by RAPD analysis, and no contamination problems were experienced, probably because, unlike most PCR methods, ours required a large amount of template DNA to generate a complete fingerprint.

RAPD fingerprinting of sequential *P. aeruginosa* isolates collected from CF patients indicated that phenotypic conversion of *P. aeruginosa* during chronic colonization is probably an adaptive response of the infecting strain, with the primary colonizing isolates up-regulating expression of MEP, down-regulating expression of pilin and flagellin, and altering their lipopolysaccharide to become serum sensitive. Approximately half (11 of 20; including patient 4 [see below]) of the patients examined were chronically colonized with a single strain of distinct genotype, and nonmotile *P. aeruginosa* isolates were recovered from eight of these patients during chronic infection (Tables 2 and 3). These data suggest that loss of flagellation and piliation occurs within individual strain types to yield the nonmotile phenotype seen uniquely in CF isolates (11). Since nonmotile *P. aeruginosa* is resistant to nonopsonic clearance by both macrophages and polymorphonuclear leukocytes (11, 12), loss of flagellation and motility may confer a survival advantage in CF. The transition to the nonmotile phenotype was not however an irreversible adaptation; nonflagellated, nonpiliated isolates collected from patient 3 (Table 2) reverted to a motile phenotype 2 years prior to the death of that patient (11).

In general, the RAPD typing data presented herein concur well with original observations obtained by RFLP analysis with the exotoxin A (13) and pilin gene probes (23), demonstrating that CF patients are often colonized with single strains of unique genotype for prolonged periods of time. However, if *P. aeruginosa* colonization of CF patients is monitored for periods greater than 2 years, more-complex epidemiologic phenomena such as coinfection or strain replacement (2, 24, 29) may be observed. We observed strain replacement in 4 of the 20 patients examined (patients 3, 15, 19, and 20; Table 2); patients 2, 6, 14, and 18 we coinfected with two or more *P. aeruginosa* RAPD types, and six different types were recovered from patient 6. *P. aeruginosa* of the same strain type was present in only 2 of the 20 patients (RAPD type 7; patients 5 and 10; Table 2; Fig. 3) and is consistent with previous observations that cross-infection at our center was rare (22).

Our study is the first to correlate the loss of motility with genomic rearrangement in isolates recovered directly from a CF patient (patient 4; Fig. 4 and 5). Loss of a single band in the RAPD fingerprint (Fig. 4) and the reduced molecular size of the smallest *Ceu*I PFGE fragment (Fig. 5) of the nonmotile isolates recovered from patient 4 suggest that a deletion of DNA may have taken place. Preliminary Southern hybridization experiments, using the 0.7-kb RAPD fragment from a type 4 strain as a probe (Fig. 4), demonstrated the presence of probe-reactive DNA in type 4 isolates which was absent in the type 4a isolates (19) and confirmed that a deletion of a portion of the *P. aeruginosa* chromosome had taken place in the genomic rearrangement. Conversion of *P. aeruginosa* to the mucoid phenotype in a rat lung model has been linked to a genetic rearrangement (30) and subsequently found to be due to insertion elements (20). We did not observe differences in the RAPD profiles of serial mucoid and nonmucoid sets of isolates (Fig. 4), which may be due to the inability of the primers we used to detect the particular insertion elements associated with conversion to mucoidy (20). The factors responsible for, and the genes lost, in the DNA deletion found in the nonmotile type 4a isolates from patient 4 remain to be fully determined. In a previous study we had demonstrated that complementation with the *P. aeruginosa rpoN* gene in *trans* may partially restore motility in these isolates (11); however, from the PFGE profile (Fig. 5), the DNA deletion appears to be substantial and may involve the loss of several loci. Despite these major alterations in both phenotype and genomic DNA, the strain persisted during chronic colonization and was subsequently recovered from patient 4 over several years.

In conclusion, we have demonstrated that expression of mucoid expolysaccharide, loss of flagellation and piliation, and acquisition of a serum-sensitive phenotype by *P. aeruginosa* probably results from adaptation of colonizing strains to chronic infection in the CF respiratory tract. We have also presented preliminary evidence that genomic rearrangements may be responsible for alteration in the motility phenotype of certain *P. aeruginosa* isolates. These data support the conclusions reached by Römmling et al. (16) that genotypically discordant *P. aeruginosa* strains develop a common phenotype during chronic CF infection. We believe that the persistence of *P. aeruginosa* during chronic CF lung infection involves different phenotypic alterations that enhance its capacity to survive in the lower respiratory tract.

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