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Serial Isolates of *Pseudomonas aeruginosa* from a Cystic Fibrosis Patient Have Identical Pilin Sequences

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Five isolates of *Pseudomonas aeruginosa* (CD2, CD3, CD4, CD5, and CD10) from a patient with cystic fibrosis were examined with regard to several genotypic and phenotypic characteristics to determine whether the patient was colonized with one or several distinct strains. Isolates CD2, CD3, and CD4 were obtained from a single sputum sample, and CD5 and CD10 were obtained 1 and 2 years later, respectively. On the basis of colonial morphology, serotyping, and antibiograms, the five isolates appeared to be different strains. However, Southern blot analysis with a 1.2-kilobase DNA probe containing the *P. aeruginosa* PAK pilin gene indicated that all five strains were identical at that genetic locus. The pilin genes of the five isolates were cloned and sequenced at the nucleotide level and found to be identical. Southern blot analysis with a probe from a separate region of the *P. aeruginosa* chromosome, a 741-base-pair *Pst*I-*Nru*I DNA fragment adjacent to the exotoxin A gene, also revealed genetic identity among these five clinical isolates. On this basis, it was concluded that this patient was colonized with a single strain of *P. aeruginosa* and that the strain had remained genetically stable over a period of 2 years. The predicted pilin sequence of the CD isolates was almost identical to that of strain PA103 (97% homology) and serologically related to PAO pilin, with which it shared 80% homology. No immunological cross-reactivity was detected between the CD and PAK pilins, which shared the least homology (62%) among the four pilins considered in this study. Although all five CD isolates contained identical pilin genes, three had acquired mutations which prevented normal expression of the pilus system. CD3 was a putative regulatory mutant which was unable to produce normal amounts of pilin, and CD4 and CD10 were putative assembly mutants which produced normal amounts of pilin but were unable to assemble the pilin subunit into intact pili.

Pseudomonas aeruginosa is a remarkably adaptive pathogen that can cause disease in plants, insects, and animals. It is the primary pulmonary pathogen in patients with cystic fibrosis (CF) and causes significant morbidity in patients with burns, cancer, and other immunosuppressive illnesses (4). Factors which can contribute to *P. aeruginosa* virulence include lipopolysaccharide (LPS) (36), alginate (49), exotoxin A (16), exoenzyme S (17), various proteases (27), hemolysin (19), phospholipase C (2), outer membrane proteins (12, 28), and polar pili (30, 50). Treatment of disease caused by this organism is difficult because of the high natural resistance of the organism to antibiotics. In patients with CF, aggressive antimicrobial therapy can help to prolong life but seldom, if ever, results in the prevention or eradication of *P. aeruginosa* infection (9).

A defect in CF host defense must exist, permitting epithelial cell adhesion, colonization, and subsequent infection by *P. aeruginosa*. Although the specific cell surface alteration that allows the adherence of *P. aeruginosa* to the mucosal epithelial surface has not been well defined, there is increasing evidence that this adherence is mediated by filamentous adhesins known as pili (25, 32, 50). These filaments are chromosomally encoded and have diameters of 5.4 nm and average lengths of 2,500 nm (10, 30). They consist of a single 15,000-dalton subunit arranged in a helix of five subunits per turn, with each turn having a 4.1-nm pitch (30, 45). *Pseudomonas* pili belong to a class of pili which are present in a wide range of gram-negative bacteria including *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Moraxella bovis*, *Moraxella nonliquefaciens*, and *Bacteroides nodosus* (8, 11, 24, 31, 35, 40). These pili are characterized by an unusual residue at the amino terminus, *N*-methylphenylalanine, followed by a highly conserved sequence of about 28 hydrophobic residues. They are generally referred to as NMePhe pili.

There is disagreement about the number of *P. aeruginosa* strains which may colonize an individual CF patient. On the basis of *P. aeruginosa* characterization by antibiotic susceptibility patterns, serotyping, pyocin typing, or bacteriophage typing, some reports have suggested that CF patients may be colonized or infected by only a single strain (41, 47). Other investigators have reported that a CF patient may be colonized by two or more different strains of *P. aeruginosa* (15, 20, 42).

Although the literature contains many descriptions of *Pseudomonas* typing methods such as colonial morphology, antimicrobial susceptibility, pyocin or phage typing, and O-serotyping (3, 13, 34, 42), these procedures are generally unreliable because of the inherent phenotypic variability of *P. aeruginosa* strains (29). Thus, Ogle et al. (29) have used a DNA probe as an epidemiological marker for *P. aeruginosa*. A 741-base-pair (bp) *Pst*I-*Nru*I fragment derived from the upstream region of the exotoxin A gene (probe U) was used as a probe in DNA hybridization experiments with *Xho*I, *Sal*I, *Bgl*II, and *Bam*HI digests of *P. aeruginosa* chromosomal DNA. Serial *P. aeruginosa* isolates from individual

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CF patients which varied in colonial morphology, serotype, and biotype were found to be identical when analyzed with the 741-bp probe. On the other hand, indistinguishable isolates (by serotyping, biotyping, and antibiograms) cultured from unrelated patients were distinguishable by using the DNA probe methodology. Thus, a DNA probe was able to eliminate much of the ambiguity associated with trying to establish relatedness among the clinical isolates morphologically.

By using techniques similar to those of Ogle et al. (29), we confirmed many of their observations with a different gene probe. A 1.2-kilobase (kb) *Hin*dIII fragment containing the PAK pilin gene (33) was used to probe over 140 *P. aeruginosa* isolates from patients with CF, burns, and cancer. Individual *Pst*I and *Hin*dIII chromosomal digestions of each isolate were performed, and the DNA was fractionated and then allowed to hybridize with the PAK pilin probe. Through these studies, we have classified 12 distinct restriction fragment patterns, whereas pilin gene cloning and sequencing studies have revealed just five unique pilin genes among the 15 clinical isolates characterized thus far (W. Paranchych, B. L. Pasloske, B. B. Finlay, and P. A. Sastry, unpublished observations). Of the 140 isolates screened, many were serial isolates from individual patients, and in most instances, the restriction fragment pattern was of only one type despite apparent differences in O serotype, colonial morphology, and antibiotic sensitivity patterns.

In this report, we present a classic example of the results of analysis of serially collected *P. aeruginosa* isolates from a single patient. Although these isolates differed with respect to colonial morphology and serotype, they contained identical pilin genes, as determined by immunoblotting with specific antipilus sera, restriction mapping using the pilin probe, and nucleotide sequencing. Of particular interest were the observations that pilin expression was turned off in one of the isolates and pilin assembly was defective in two others. These observations are discussed in regard to *P. aeruginosa* strain heterogeneity in individual colonized patients and the preferred use of genetic probes for *Pseudomonas* typing rather than those which monitor phenotypic characteristics of the bacterium.

MATERIALS AND METHODS

Bacterial strains. *P. aeruginosa* CD2, CD3, CD4, CD5, and CD10 were isolated from a single CF patient in Vancouver, British Columbia, Canada. The relevant information for each strain is given in Table 1. *P. aeruginosa* PAK and PAO and *Escherichia coli* JM83 have been described elsewhere (33, 38).

Preparation of rabbit antisera. Antipilus sera were prepared with purified pili from *P. aeruginosa* PAK and PAO as previously described (39).

SDS-PAGE and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Laemmli (21), using 15% polyacrylamide running gels. Whole-cell bacterial lysates (3×10^7 bacteria) were subjected to SDS-PAGE, after which the separated proteins were electrophoretically transferred to nitrocellulose sheets (43). The excess protein-binding capacity was blocked with bovine serum albumin, and the sheets were allowed to react sequentially with either PAK or PAO rabbit antipilus serum (1/1,000 dilution) and [^{125}I]protein A (46). Detection of bound radiolabeled protein A was accomplished by autoradiography of washed, dried sheets.

Phage sensitivity assay. The CD isolates were streaked on Luria-Bertani (LB) agar, and 2 μl of the pilus-specific bacteriophage PO4 (5) was pipetted onto the cells. The cells were allowed to grow overnight at 37°C. A zone of clearing within the grown cells indicated lysis and the assembly of retractable pili.

Electron microscopy. A loopful of cells freshly grown on an LB agar plate was suspended in 50 μl of phosphate-buffered saline (pH 7.0), and 5 μl of the suspension was placed for 30 s on a copper grid coated with a hydrophilic carbon film. After being washed two times with phosphate-buffered saline, the grid was stained with 0.5% sodium phosphotungstate and examined with a Philips EM420 electron microscope.

Recombinant DNA techniques. Chromosome isolation, digestion with restriction endonucleases, nick translations, and the rapid isolation of plasmid DNA were performed as described previously (33). DNA hybridizations were carried out under high-stringency conditions (23) except that washing of the nitrocellulose filters was done at 21 rather than 65°C. Cloning the pilin genes involved complete digestion of the chromosomal DNA with *Hin*dIII and then ligating it into pUC8. The chimeras were transformed into *E. coli* JM83, and the colonies containing inserts were screened by using the nick-translated 1.2-kb *Hin*dIII PAK pilin gene probe (33). Large preparations of plasmid DNA from the positive clones were made by CsCl density gradient centrifugation (7). Nucleotide sequencing of these clones were performed by the dideoxy method of Sanger et al. (37), using M13mp19 and a 26-bp universal primer (26).

Exotoxin A probe. The chimera ptoxETA (14) was generously provided by M. Vasil, Department of Microbiology and Immunology, University of Colorado Center for the Health Sciences, Denver. Probe U (44), obtained by a *Pst*I-*Nru*I digestion of ptoxETA, was used for the DNA hybridizations.

Antibiotic susceptibility testing. Susceptibility to various antibiotics was tested by both broth dilution and agar disk diffusion methods. Broth dilution was performed with M.I.C.-Concept (Microtech Medical Systems, Inc., Aurora, Colo.) for amikacin, gentamicin, ticarcillin, and tobramycin, following the recommendations of the manufacturer. Disk diffusion was performed with commercial disks for amikacin, aztreonam, carbenicillin, ciprofloxacin, cotrimoxazole, gentamicin, piperacillin, ticarcillin, and tobramycin. Susceptibilities were determined based on zone sizes recommended by the manufacturer.

RESULTS

Phenotypic characteristics of *P. aeruginosa* isolates from a patient with CF. The colonial morphology, LPS serotyping, and antibiograms of the five *P. aeruginosa* isolates from a single CF patient are given in Tables 1 and 2 and indicate some obvious phenotypic differences.

Of the three different colonial types (CD2, CD3, and CD4) picked from a single sputum samples obtained from this patient on 15 February 1982, the serotype could be determined only for the mucoid strain since the dwarf and rough colonial types proved to be autoagglutinable. The proportions of mucoid and nonmucoid strains in this and subsequent sputum samples were not determined. Samples obtained approximately 1 and 2 years later were mucoid (CD5) and nonmucoid (CD10), respectively. The mucoid strain was again found to be serotype 11, and the nonmucoid strain was

TABLE 1. Characteristics of several *P. aeruginosa* isolates from a patient with CF

Strain[a]	Data isolated	Colony morphology	LPS serotype[b]	Pilin serotype[c]
CD2	2-15-82	Mucoid	11	PAO
CD3	2-15-82	Dwarf	AA	NA
CD4	2-15-82	Rough	AA	PAO
CD5	2-24-83	Mucoid	11	PAO
CD10	2-21-84	Classic	3	PAO

[a] All strains had restriction fragment pattern 1 based on *Pst*I and *Hin*dIII fragments which hybridized to the 1.2-kb *Hin*dIII pilin gene probe from *P. aeruginosa* PAK (33).

[b] LPS serotyping was done with the International Antigenic Typing System (Difco Laboratories, Detroit, Mich.). AA, Autoagglutinable.

[c] Determined by immunoblotting with polyclonal antiserum. NA, Not applicable.

serotype 3. With respect to the antibiograms, only strains CD4 and CD10 were identical; therefore, for the five isolates there were four different antibiograms. These data suggest that this patient was colonized with at least two different strains of *P. aeruginosa*.

Immunological properties of CD pilin. Whole-cell immunoblots of the five CD isolates were performed using either PAK or PAO antipilus serum. All of the isolates expressed CD pilin which cross-reacted well with PAO antipilus serum, except CD3 pilin, which was not detected by either serum (Fig. 1). The PAO antipilus serum exhibited weak cross-reactivity with PAK pilin, but there was no detectable cross-reactivity of PAK antipilus serum with PAO or CD pilin.

Analysis of genomic DNA by Southern hybridization with the PAK pilin gene probe. When the chromosomal DNA preparations from all five isolates were subjected to restriction enzyme digestion and hybridization with a 1.2-kb *Hin*dIII fragment containing the PAK pilin gene, nearly identical patterns were observed (Fig. 2), indicating homology of the DNA of the isolates with the PAK pilin gene and its flanking sequences and strongly suggesting closely related pilin genes among the five isolates themselves.

Cloning and sequencing of the CD pilin genes. Cloning of the pilin genes from the five CD strains listed in Table 1 was based on a strategy similar to that described by Pasloske et al. (33) since the CD pilin genes were also located within 1.2-kb *Hin*dIII fragments. The pilin gene sequence, which was identical among all five of the CD isolates, is shown in Fig. 3. Repetitive cloning and sequencing of each isolate provided a high level of confidence in the observed DNA sequences.

Extent of piliation of CD strains. Piliation of the CD strains was monitored by electron microscopy and bacterial sensitivity to the pilus-specific bacteriophage PO4 (5). Pili were visualized by electron microscopy on strains CD2 and CD5 but not on strain CD3, CD4, or CD10 (Table 3). These observations were supported by PO4 sensitivity tests, which showed that strains CD2 and CD5 were sensitive to this pilus-specific phage, whereas strains CD3, CD4, and CD10 were phage resistant.

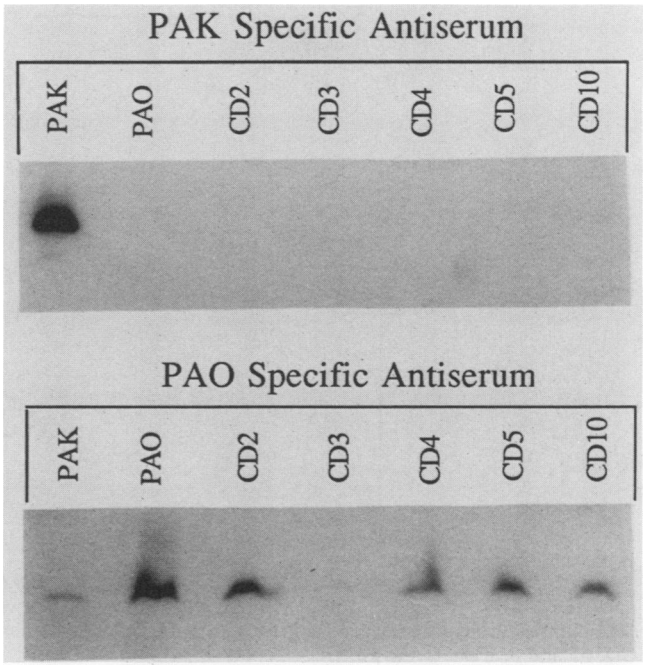

FIG. 1. Autoradiograph of an SDS-PAGE immunoblot showing reactivity of pilin in whole-cell bacterial lysates from seven strains of *P. aeruginosa* with PAK and PAO antipilus polyclonal rabbit antisera. [^{125}I]protein A was used to indicate the positions of antibody-bound protein. The equivalent of 3×10^7 bacteria was applied to each lane for SDS-PAGE.

Analysis of genomic DNA by Southern hybridization with the *Pst*I-*Nru*I exotoxin A gene probe. The *Pst*I-*Nru*I exotoxin A gene probe (probe U) (44) appears to be a sensitive indicator of *P. aeruginosa* strain differences, since Ogle et al. (29) were able to distinguish more than 100 different strains. We thus analyzed the CD strains with this probe to determine whether it could detect genomic differences that were not evident with the pilin gene probe. Chromosomal DNA preparations from each of the CD strains and strain PAK were digested with *Sal*I and *Bam*HI and subjected to Southern hybridization with probe U. The CD strains yielded identical restriction polymorphism patterns (Fig. 4). The six strains for which results are shown in Fig. 4 were also probed with the *Pst*I-*Nru*I fragment after digestion of

TABLE 2. Antibiograms of several *P. aeruginosa* isolates from a patient with CF[a]

Strain	Response to:								
	Amikacin	Aztreonam	Carbenicillin	Ciprofloxacin	Gentamicin	TMP-SMX	Piperacillin	Ticarcillin	Tobramycin
CD2	S	MS	S	S	S	S	S	S	S
CD3	S	S	S	S	S	S	S	S	S
CD4	MS	S	S	S	MS	R	S	S	S
CD5	MS	S	S	S	MS	S	S	S	S
CD10	MS	S	S	S	MS	R	S	S	S

[a] Antibiotic susceptibility was determined by agar disk diffusion and/or broth dilution (M.I.C.-Concept; Microtech Medical Systems, Inc.) techniques. S, Susceptible; R, resistant; MS, moderately susceptible. TMP-SMX, Trimethoprim-sulfamethoxazole (Septra; Burroughs Wellcome Co., Research Triangle Park, N.C.).

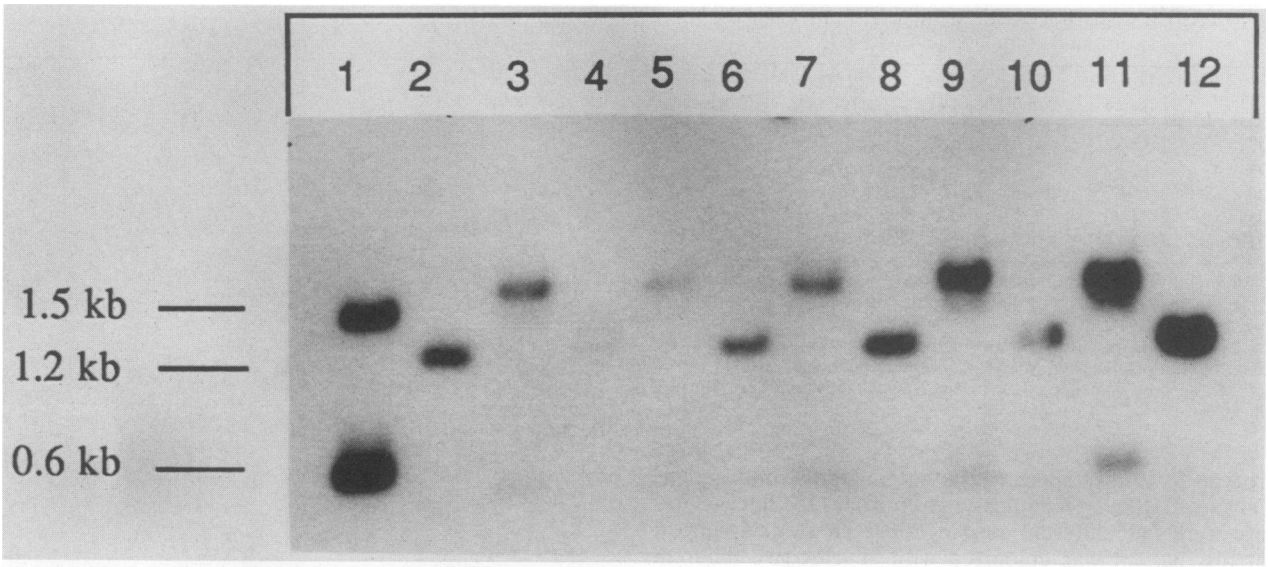

FIG. 2. Restriction digests of *P. aeruginosa* chromosomal DNA probed with a nick-translated 1.2-kb *Hin*dIII fragment containing the *P. aeruginosa* PAK pilin gene. The genomic DNAs were digested with *Pst*I and *Hin*dIII and hybridized with a 1.2-kb PAK pilin gene probe. Odd-numbered lanes contained *Pst*I digests. Even-numbered lanes contained *Hin*dIII digests. Lanes: 1 and 2, PAK; 3 and 4, CD2; 5 and 6, CD3; 7 and 8, CD4; 9 and 10, CD5; 11 and 12, CD10.

chromosomal DNA with *Pst*I and *Hin*dIII (data not shown). These patterns were also identical. Thus, the restriction fragment lengths were identical in CD and PAK strains whether tested with probe U or the pilin gene probe.

DISCUSSION

P. aeruginosa strains are able to change many surface characteristics to adapt to different environmental niches. These modifications evidently do not involve extensive chromosomal mutations but are due almost entirely to phenotypic alterations in the expression of surface macromolecules such as LPS, alginate, or outer membrane proteins (6). These changes lead to altered colonial morphology, serotypes, phage and pyocin susceptibility patterns, and antibiograms (6, 29). In the past, differences in these phenotypic properties were usually construed as evidence for unique strains of *P. aeruginosa* (15, 20). However, this study and that of Olge et al. (29) suggest that these phenotypic traits may provide misleading information with regard to epidemiological typing of *Pseudomonas* strains, whereas the use of DNA probes for specific regions of the *P. aeruginosa* chromosome appears to provide a reliable approach to strain typing.

We examined five isolates of *P. aeruginosa* from a CF patient with regard to various phenotypic and genotypic characteristics to determine whether the patient was colonized with one or several strains. The isolates were selected initially on the basis of observed differences in colonial morphology, serotype, and antibiotic resistance patterns, which indicated that this patient may have been colonized with several unique *Pseudomonas* strains. However, Southern blot analysis with a 1.2-kb *Hin*dIII DNA probe containing the *P. aeruginosa* PAK pilin gene suggested that genetically all five strains were closely related. The pilin genes of all five isolates were then cloned and sequenced at the nucleotide level and found to be identical. Moreover, Southern blot analysis with a probe from a completely different region of the *P. aeruginosa* chromosome, the 741-bp *Pst*I-*Nru*I pre-exotoxin A probe (probe U) used by Ogle et al. (29), also showed identity among these clinical isolates. These observations provided compelling evidence that all five isolates were clonally related and suggested that this patient was colonized with a single strain of *P. aeruginosa*. Additional studies in this laboratory (W. Paranchych, K. Volpel, D. P. Speert, and M. Campbell, unpublished studies) and that of Ogle et al. (29) of serial isolates from other patients yielded similar observations. It thus appears that in most instances, CF patients are colonized with a single strain of *P. aeruginosa*, although examples of colonization with more than one strain may yet be found.

Three of the CD isolates examined in the present study seemed to have acquired mutations which prevented normal expression of the pilus system. CD3 was a putative regulatory mutant which was unable to produce normal amounts of pilin, and CD4 and CD10 were putative assembly mutants which produced normal amounts of pilin but were unable to assemble the pilin subunits into intact pili. This suggests that after initial colonization, *P. aeruginosa* strains do not need to produce pili to continue colonizing the respiratory tracts of CF patients. It would be undesirable for any typing system to be sensitive to subtle genetic or phenotypic changes of this type since they do not reflect the overall genotype of the strain.

Two *P. aeruginosa* DNA probes are now available for genotypic analysis, probe U reported by Ogle et al. (29) and the pilin gene probe described in this report. Extensive studies are in progress to assess the number of restriction pattern polymorphisms that will be found among *Pseudomonas* isolates with each of these probes, and other probes will undoubtedly be reported in the near future. Ogle et al. (29) have distinguished more than 100 genetically different isolates of *P. aeruginosa* with their probe, although they did not state the total number of isolates screened. Thus, there seems to be considerable restriction fragment heterogeneity among *P. aeruginosa* strains, as determined with their probe.

To date, we have used the pilin DNA probe to screen approximately 140 *Pseudomonas* strains from patients with

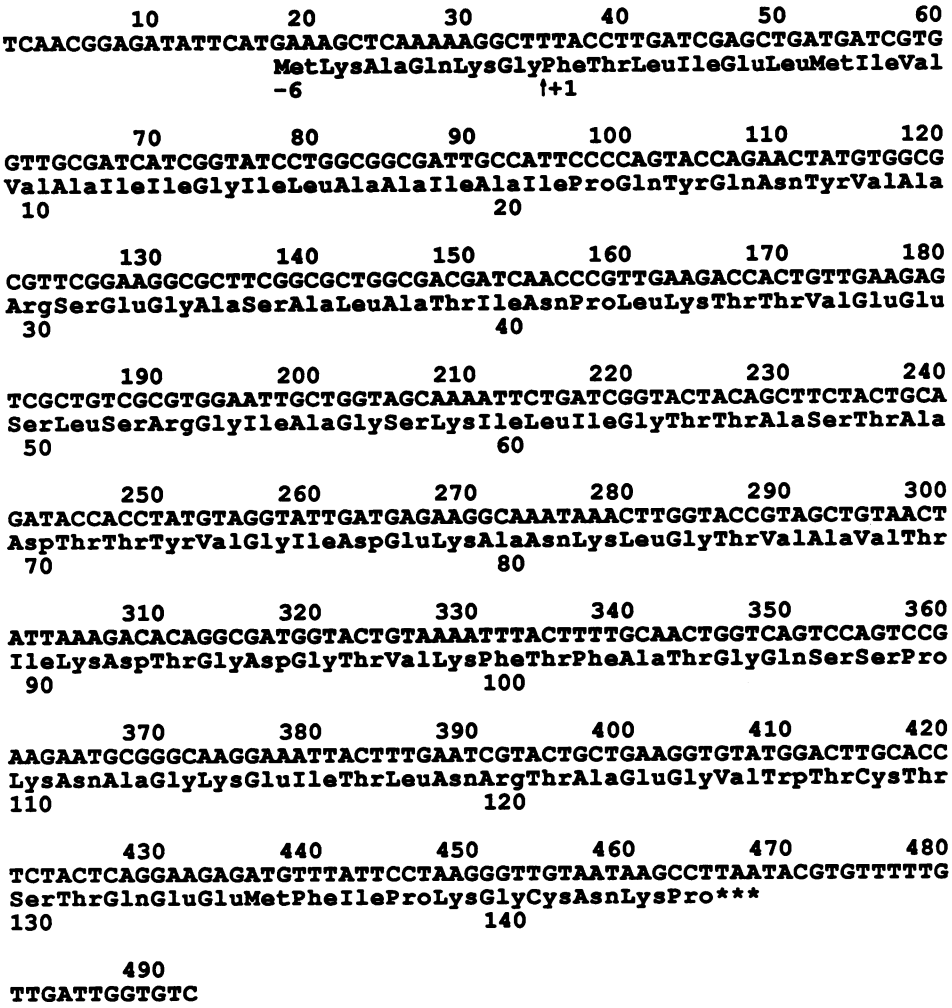

FIG. 3. Nucleotide sequence of a 492-bp region containing the pilin gene of five CD strains of *P. aeruginosa*. The nucleotide sequence was identical in all five CD strains (CD2, CD3, CD4, CD5, and CD10). The precursor *P. aeruginosa* pilin protein sequence with a leader sequence is shown, with the putative cleavage site of the leader sequence indicated by an arrow. The numbers above each row refer to nucleotide positions. The numbers below each row refer to amino acid positions. ***, Stop codon.

CF, burns, and cancer. Twelve unique restriction fragment patterns have been detected thus far, suggesting that the total number of restriction fragment patterns detectable with this probe is limited. The pilin gene probe thus appears to be well suited for epidemiological investigations. We are also in the process of delineating the total number of unique pilin sequences and preparing specific DNA probes for each type of pilin gene, as well as specific antiserum for each pilus type. These antisera and specific DNA pilin gene probes will provide additional tools for strain typing which will avoid the disadvantages associated with the use of phenotypic properties.

TABLE 3. Pilus expression by several *P. aeruginosa* isolates from a patient with CF

Strain	Pilin biosynthesis[a]	Presence of pili on cell surface	
		Electron microscopy	Phage PO4 sensitivity
CD2	+	+	+
CD3	−	−	−
CD4	+	−	−
CD5	+	+	+
CD10	+	−	−

[a] Detected by whole-cell immunoblot analysis.

It is important to distinguish between the restriction fragment patterns, which depend on the locations of *Pst*I and *Hin*dIII recognition sites in the flanking sequences of the pilin gene, and the sequence of the pilin gene within the restriction endonuclease fragment. Although we have thus far detected 12 distinct restriction fragment polymorphisms among the strains screened, we have identified only five unique pilin gene sequences and four unique pilus serotypes in the 15 *P. aeruginosa* pilin genes sequenced. It is of interest that the CD strains yielded a PAK-like restriction fragment polymorphism, but the pilin turned out to be immunologically related to PAO pilin. Since strain PAO chromosomal DNA yields a different restriction fragment pattern, it is evident that the restriction fragment pattern, which depends on the locations of *Pst*I and *Hin*dIII restriction sites in the flanking sequences of the pilin gene, need not reflect the type of pilin gene actually present in the fragment. That the CD pilin gene is significantly different from that of strain PAK is evident in Fig. 2, in which it can be seen that

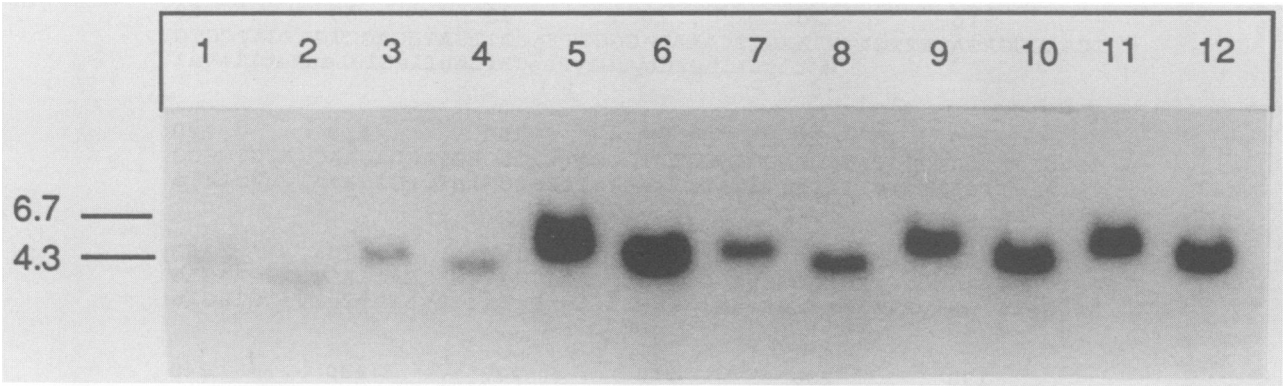

FIG. 4. Restriction digests of *P. aeruginosa* chromosomal DNA probed with a labeled 741-bp *Pst*I-*Nru*I exotoxin A probe (44). The genomic DNAs were digested with *Sal*I and *Bam*HI and hybridized with a 741-bp pre-exotoxin A probe. Odd-numbered lanes contained *Bam*HI digests. Even-numbered lanes contained *Sal*I digests. Lanes: 1 and 2, PAK; 3 and 4, CD2; 5 and 6, CD3; 7 and 8, CD4; 9 and 10, CD5; 11 and 12, CD10. The numbers in the margin indicate the positions of standard fragments (6.7 and 4.3 kb) which migrated at positions similar but not identical to those of fragments detected with the pre-exotoxin A probe.

the 600-kb *Pst*I fragment from the CD strains hybridized more weakly with the PAK pilin gene probe than did the same fragment from PAK chromosomal DNA. Interestingly, the sizes of the restriction fragments from the CD strains were virtually identical to those from our prototype PAK strain with both the pilin gene probe and probe U, but the actual pilin gene was quite different from that of strain PAK, indicating the usefulness of pilus serotyping in addition to restriction mapping to identify specific genotypes. Also, it is important that nearly identical patterns were observed among the five strains (Fig. 2), indicating homology of the DNA of the five isolates with the PAK pilin gene and its flanking sequences and strongly suggesting closely related pilin genes among the five isolates themselves. However, the intensities of the bands identified in the CD DNA were generally weaker than those in the homologous PAK DNA. This indicated that detectable differences in DNA homology existed between the CD and PAK pilin genes even though the *Pst*I and *Hin*dIII restriction sites flanking the pilin genes were identical. It is also worth mentioning that relative intensities of bands may be misleading because anomalies may arise due to gel "smiling," differences in the amounts of DNA loaded on the gel, and the efficiency of DNA transfer to the nitrocellulose. Thus, it is important to emphasize the relative sizes of the restriction fragments in this method of analysis rather than the relative intensities of the bands.

The CD pilin protein sequence was almost identical to that of the PA103 pilin (97% homology) (Fig. 5), which was also isolated from human sputum (18, 22). These two proteins had only four conservative amino acid differences, which correlate with the only four nucleotide differences between the two genes. The homology between the CD and PAO pilin proteins, which are serologically related, was somewhat less (80% homology), whereas that between the CD/PA103 and PAK pilins was significantly lower (62% homology). The extent of homology between the PAK and PAO pilins was 65%. Because of their close similarity, the CD and PA103 pilin proteins can be considered to be of the same unique sequence type. The PAK and PAO pilins would be classified as two other unique protein sequences.

It was evident from the immunoblot that the pilins of CD2, CD4, CD5, and CD10 were immunologically related to PAO pilin but not PAK pilin. The fact that no pilin was detected in the CD3 cell extract (Fig. 1) suggested one of three possibilities: (i) CD3 cells were defective in the expression of CD-like pilin, (ii) they produced normal amounts of a pilin of a different serotype, or (iii) the pilin was rapidly degraded. Because the CD3 pilin gene was identical to the other CD pilin genes, it is likely that the CD3 pilin was not detected due to either the first or third possibilities.

Since CD pilin cross-reacted with PAO antipilus serum, it is likely that the immunodominant domain in the CD pilin is closely related to that in the PAO pilin, suggesting similar or identical amino acid sequences in this region. The amino acid sequences in Fig. 5 show two possible regions for the cross-reactive epitope(s) between the CD and PAO pilins. The sequences which are identical in the CD and PAO pilins but different in the PAK pilin encompass positions 55 to 69 and 88 to 105. These predicted antigenic determinants are within the boundaries determined by previous studies, which localized the major antigenic determinant of the PAK pilin to within residues 70 to 110 (39) and that of the PAO pilin to within residues 54 to 121 (P. A. Sastry and W. Paranchych,

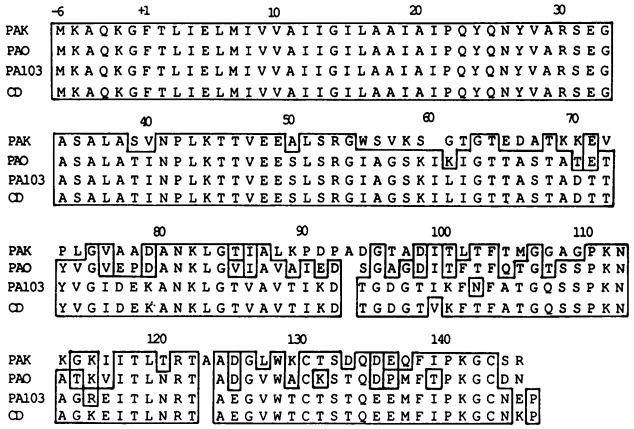

FIG. 5. Comparison of the amino acid sequences of the PAK, PAO, PA103, and CD pilin polypeptides. The amino acid sequences were aligned by visual examination and matching of amino acid residues on the basis of amino acid similarity values obtained from Bacon and Anderson (1). Empty spaces were generated to allow for the best possible alignment. Boxed areas represent regions of homology. The PAK and PAO sequences were obtained from the data of Pasloske et al. (33) and Sastry et al. (38), respectively. The PA103 sequence was obtained from Johnson et al. (18).

unpublished results). On the basis of the sequence comparison and these experimental results, one of the major antigenic determinants may be comprised of residues 88 to 105.

Strains of *P. aeruginosa* from CF patients are a unique class of bacteria; many of the isolates are mucoid and serum sensitive and possess rough O antigens in their LPSs. Conventional *Pseudomonas* typing systems are based upon serological reactions with O antigens. Consequently, many CF *Pseudomonas* isolates are nontypeable. This has frustrated attempts to characterize these strains for epidemiological purposes. The genetic analysis with the pilin gene probe described in this report has proven capable of characterizing all the CF *Pseudomonas* strains studied to date (more than 140 isolates). Pilin gene probe analysis of other strains of *P. aeruginosa* are being conducted to gain insights into the natural history of *Pseudomonas* colonization and infection in patients with CF. Since most patients appear to be colonized with a single *Pseudomonas* genetic type, it should be possible to answer questions regarding the risk of patient-to-patient spread and the source of initial colonization.

ACKNOWLEDGMENTS

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