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Conversion of *Pseudomonas aeruginosa* to the Phenotype Characteristic of Strains from Patients with Cystic Fibrosis

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Isolates of *Pseudomonas aeruginosa* from cystic fibrosis patients are unusual; they are often susceptible to the bactericidal effect of human serum, have a rough lipopolysaccharide, and produce an exopolysaccharide that is responsible for the characteristic mucoid phenotype. In contrast, strains from the environment and from patients with other diseases usually have smooth lipopolysaccharide, do not produce very much mucoid exopolysaccharide, and are phenotypically nonmucoid. The predominance of mucoid strains of *P. aeruginosa* in infections of patients with cystic fibrosis has not been explained. In the lower airways, where *P. aeruginosa* persists in cystic fibrosis, nutrients for bacterial growth may be limited. We investigated whether growth of *P. aeruginosa* under conditions of suboptimal nutrition causes conversion to the characteristic cystic fibrosis phenotype. Ninety-two strains of *P. aeruginosa* were maintained for up to 90 days in a minimal medium with acetamide as the sole carbon source. In 56 (52%) of 107 cultures, isolates with rough lipopolysaccharide emerged, and in 20 (19%) of 104 nonmucoid cultures, mucoid isolates were recovered. Strains with rough lipopolysaccharide also were sensitive to the bactericidal effect of normal human serum. Under conditions of suboptimal nutrition in vitro, isolates of *P. aeruginosa* emerged that produced rough lipopolysaccharide and were mucoid, typical of many isolates from cystic fibrosis patients. This peculiar phenotype may arise as a consequence of nutritional limitation within the cystic fibrosis respiratory tract rather than from features unique to these strains of bacteria.

Strains of *Pseudomonas aeruginosa* from patients with cystic fibrosis (CF) are an unusual class of microorganisms. They often differ from other *P. aeruginosa* isolates by virtue of their mucoid colonial phenotype, which derives from the production of copious amounts of a mucoid polyuronic acid exopolysaccharide (9). *P. aeruginosa* isolates from patients with CF also frequently possess a rough lipopolysaccharide (LPS) (12), which lacks the normal O-polysaccharide side chain that protects gram-negative bacteria from the bactericidal effect of human serum. Consequently these strains are often serum sensitive (12, 15, 29, 34, 40) and nontypable with the conventional antisera directed against the O polysaccharide of LPS (16, 32, 37).

A number of theories have been offered to explain the preponderance of these peculiar *P. aeruginosa* strains in CF respiratory secretions. Selection of mucoid strains under the pressure of antibiotic therapy has been suggested (11). However, mucoid strains are often more susceptible to antibiotics than their nonmucoid isogenic variants (11, 12). Nosocomial transmission has also been proposed (16, 30, 45), but there is no compelling evidence that this occurs (37, 39, 41). Furthermore, some patients are colonized with mucoid *P. aeruginosa* at the first presentation to a CF clinic, before having had contact with another patient with CF (Speert, unpublished observation). The exquisite serum sensitivity of these strains, due to their rough LPS (12), is particularly difficult to understand in view of their capacity to survive in the presence of an inflammatory response in respiratory secretions.

In CF, colonization and infection by *P. aeruginosa* occur in the endobronchial space. This is quite different from the invasive type of infection caused by *P. aeruginosa* in other disease states (e.g., septicemia in neutropenic hosts and wound infections after thermal injury). In the endobronchial space, bacteria probably are deprived of the abundant nutrients available during invasive disease. The purpose of these investigations was to determine whether nonmucoid and smooth-LPS strains of *P. aeruginosa* acquire the phenotypic characteristics of CF strains under conditions of suboptimal nutrition.

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MATERIALS AND METHODS

Strains. All *P. aeruginosa* strains were serotyped by using the International Antigenic Typing System (Difco Laboratories, Detroit, Mich.), in which the bacteria are agglutinated by antisera directed at the O polysaccharide of LPS (37). Strains were identified as *P. aeruginosa* by conventional microbiological methods (37) and were characterized by colonial morphology as mucoid, classic, enterobacterium-like, rough, or dwarf (42).

P. aeruginosa strains from both clinical and environmental sources were studied (Table 1). Each strain was typed with a single O-polysaccharide-specific antiserum (presumptive evidence of the presence of smooth LPS). The 10 CF strains, obtained from different patients cared for at the Cystic Fibrosis Assessment Clinic, British Columbia's Children's Hospital, were atypical for CF isolates and were chosen for the study because they could be typed easily and had smooth LPS. Of the 10 CF isolates, 3 were of mucoid morphotype, 3 were dwarf, and 4 were classic. Clinical isolates from different patients without CF were kindly provided by John Anderson, British Columbia's Children's Hospital, and John Smith, Vancouver General Hospital (Table 1).

TABLE 1. Strains of *P. aeruginosa*

Source	No. of strains	Strain identification
Clinical		
CF sputum	10	C124, C132, C192, C253, C315, C395, C494, C632, C749, C1029
Surface[a]	9	V24, V29, V42, V81, V83, V100, V113, V116, ENV96
Blood	6	V5, V76, V106, V110, V132, V133
Urine	5	V2, V15, V22, V64, V73
Stool	3	V26, V88, V101
Wound	2	V34, V93
Cyst	1	V41
Placenta	1	V39
Throat[b]	2	V75, V108
Environmental		
Sink or shower drain	12	ENV3, ENV23, ENV53, ENV73, ENV94, ENV95, ENV105, ENV112, ENV115, ENV118, ENV128, ENV144
Vegetable	10	ENV1, ENV2, ENV4, ENV5, ENV6, ENV7, ENV8, ENV9, ENV10, ENV11
Air	1	ENV13
River water	30	ENV36, ENV37, ENV38, ENV39, ENV41, ENV42, ENV43, ENV46, ENV47, ENV48, ENV49, ENV50, ENV51, ENV52, ENV53, ENV54, ENV56, ENV57, ENV58, ENV59, ENV60, ENV61, ENV62, ENV63, ENV64, ENV65, ENV66, ENV67, ENV68, ENV69

[a] From cultures of uninfected skin, ears, and other superficial sites.
[b] From surveillance cultures of neonates and neutropenic children.

Environmental isolates of *P. aeruginosa* from river water were obtained from Robert Hancock (University of British Columbia) and were originally isolated in Japan (21). These strains were of serotypes 1, 3, 4, 5, 6, 8, 9, 11, 15, and 16. The isolate from the air has been described previously (37). The other environmental isolates were obtained as follows. Sink or shower drains were scrubbed with rayon-tipped swabs and then placed in acetamide broth (18), composed of 20.0 g of acetamide (BDH Chemicals, Toronto, Ontario), 5.0 g of NaCl, 1.0 g of $NH_4H_2PO_4$, and 1.0 g of K_2HPO_4 in 1 liter of distilled water with 10 ml of 1 M $MgSO_4$. Pieces of vegetables (parsley, onion, lettuce, potato, and green pepper) were also inoculated into acetamide broth. These cultures were incubated at 37°C for 7 days and then subcultured onto PC agar (5), a selective medium for *P. aeruginosa* composed of Columbia agar with 30.0 g of phenanthroline per liter and 30.0 g of C-390 (Norwich Eaton Pharmaceuticals, Norwich, N.Y.) per liter, for 24 to 48 h.

Multilocus enzyme electrophoresis. All isolates showing a change to rough LPS or to the mucoid phenotype were examined for electrophoretically demonstrable allelic variation at 18 loci by methods described previously (22, 35). Each unique profile of electromorphs (allozymes) for the 18 enzymes was recognized as a distinctive electrophoretic type.

Cultivation in acetamide broth. The 92 strains of *P. aeruginosa* were cultured in acetamide broth for 30 to 90 days. In all, 107 cultures were established (14 strains were cultured twice or more). Approximately 10^6 CFU in 1 ml were inoculated into 10 ml of acetamide broth and maintained under static conditions at 37°C in 15-ml screw-cap glass tubes. Every 30 days, the cultures were sampled carefully from the pellicle or the air-liquid interface, taking care not to agitate the culture, and were subcultured on Columbia agar. Isolates with different colonial morphology were subcultured, serotyped with LPS-specific antisera, and frozen.

Six strains (three clinical isolates [V75, V76, and V81] and three environmental isolates [ENV2, ENV8, and ENV10]) were cultured again in acetamide broth and were sampled at more frequent intervals for up to 120 days for enumeration of viable counts. These culture tubes were vortexed vigorously before their contents were sampled to ensure accurate quantitation of viable bacteria.

Analysis of LPS. LPS was extracted from six of the smooth-LPS strains of *P. aeruginosa* (V75, V76, V81, ENV2, ENV8, and ENV10) and from isolates derived from these six after prolonged cultivation in acetamide broth. Some of the derived strains (referred to as progeny) were agglutinated by multiple LPS-specific antisera. The LPS was extracted and analyzed by silver-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis by the method of Hitchcock and Brown (14).

Serum sensitivity testing. Twenty strains of *P. aeruginosa* and their progeny were tested for susceptibility to the bactericidal effect of serum as described previously (37). Approximately 10^6 CFU of each strain were incubated in 10 or 25% human serum (pooled from five healthy adults and diluted in Tris buffer [pH 7.4] with 0.1% gelatin). Simultaneous control assays were performed in Tris buffer without added serum. Viable counts were determined by the spread-plate technique before and 120 min after the bacteria were inoculated into the serum.

Extraction and analysis of mucoid material. Mucoid extracellular material was extracted from an isolate that converted to the mucoid morphotype after in vitro cultivation of strain ENV48 as described previously (9). Briefly, the bacteria were grown at 37°C for 24 h on tryptose blood agar, without added blood. Bacteria and extracellular slime were scraped from the agar into sterile normal saline, stirred until uniform, and centrifuged repeatedly until a pellet was no longer visible. The mucoid material was precipitated from the supernatant by adding 95% ethanol at a ratio of three parts to one. The precipitate was spooled out with a glass rod and air dried. The uronic acid content of this material was determined by the carbazole assay (20). It was compared with the known standard polyuronic acid polysaccharides, mucoid exopolysaccharide from a strain of mucoid *P. aeruginosa* (a gift from Alfred Linker, Department of Pathology, University of Utah), and sodium alginate (Sigma Chemical Co., St. Louis, Mo.).

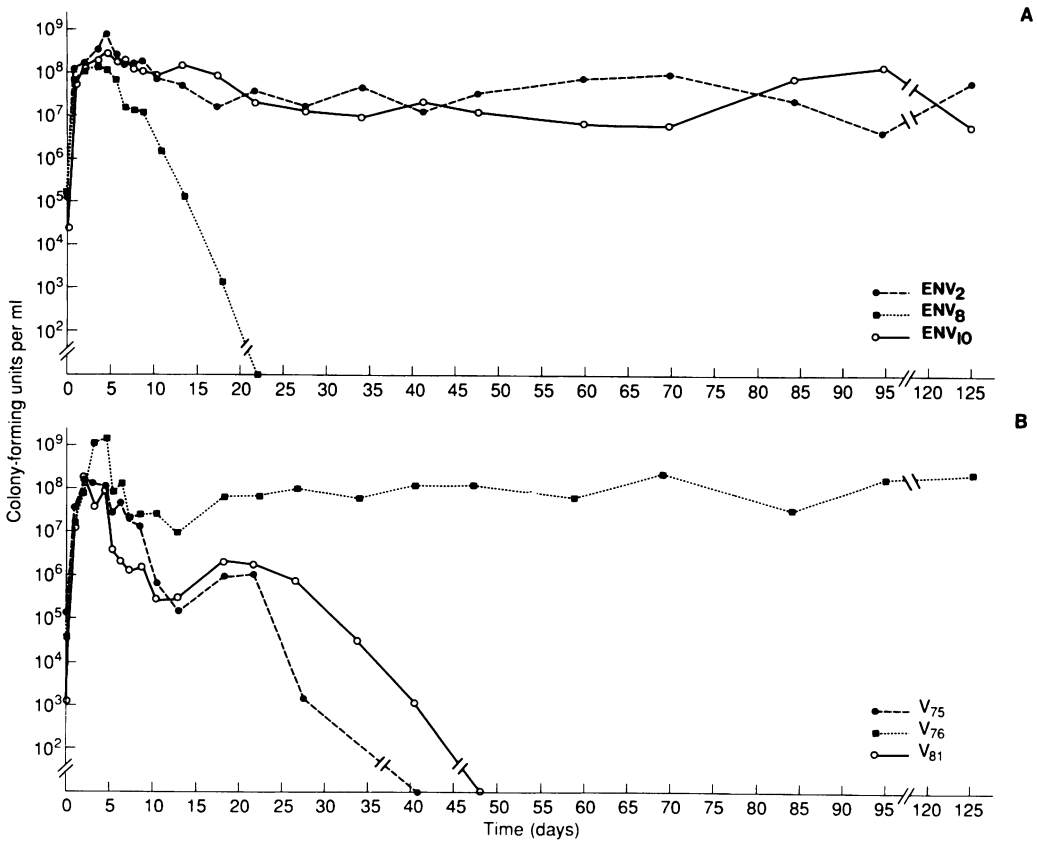

FIG. 1. Viability of three environmental (A) and three clinical (B) strains of *P. aeruginosa* during prolonged incubation in acetamide broth.

RESULTS

Survival of *P. aeruginosa* during prolonged cultivation in acetamide broth. In all, 107 cultures of the 92 strains were established; all strains remained viable after 30 days, 66 (61.6%) were viable at 60 days, and 23 (21.4%) were viable at 90 days. There was no evidence of contamination by other bacterial species.

The viability of six of these strains (three CF and three environmental) was assessed at more frequent intervals (Fig. 1). The cultures were maintained under static conditions but were vortexed before being sampled. All six strains remained viable for more than 2 weeks, and three (V76, ENV2, and ENV10) were still viable until the cultures dried out at 125 days. These conditions were probably less conducive than static conditions to sustaining viability; there was repeated vortexing with frequent aeration and therefore more rapid growth of *P. aeruginosa*, a strict aerobe. The bacteria may have exhausted available nutrients more rapidly than if they had remained static.

Phenotypic dissociation of *P. aeruginosa* during cultivation in acetamide broth. Multiple colonial morphotypes were observed in most of the cultures after incubation for 30 days or more in acetamide broth. This colonial dissociation has been observed previously (44), but the emergence of mucoid colonies was unexpected. Up to four morphotypes (classic, dwarf, enterobacter, rough, and mucoid) were observed in each of the cultures, and 20 (19.2%) of the original 104 nonmucoid cultures gave rise to mucoid colonial variants. Emergence of the mucoid phenotype occurred among environmental (14 [24.6%] of 57), CF (2 [14.3%] of 14), and other clinical (4 [12.1%] of 33) strains. Examples of morphotypic dissociation are shown in Table 2 for two CF, five environmental, and three other clinical isolates. There was considerable instability among the five colonial morphotypes, with new variants arising and then disappearing on subsequent sampling (e.g., the mucoid morphotypes from strains ENV105 and ENV2). Figure 2 depicts a classic type environmental isolate from river water (ENV48) and a mucoid variant derived by cultivation in acetamide broth for 30 days. The mucoid strain had the characteristic slimy, glistening appearance with coalescence of adjacent colonies. In contrast, the individual colonies of strain ENV48 were discrete and had the characteristic feathered edges of the classic morphotype.

The LPS-specific serotype changed from monotypable to polytypable or nontypable in 56 (52.3%) of 107 cultures after cultivation for 30 days or more in acetamide broth. This was interpreted as preliminary evidence for conversion from smooth to rough LPS, since the antisera react with the O-polysaccharide moiety of smooth LPS. Polytypability was due, presumably, to the fact that several of the antisera react with common antigenic determinants on the exposed core polysaccharide of rough LPS. Examples of changes in LPS serotype during prolonged cultivation are shown in Table 2. After 30 days or more, some strains became polytyable, some became nontypable (C632) or autoagglutinable (ENV23), and others demonstrated an apparent change in serotype from one single type to another in part of the population (ENV105 and V76). The acquisition of a new serotype probably resulted from the loss of the O polysac-

TABLE 2. Phenotypic conversion of several strains of *P. aeruginosa* during prolonged culture in acetamide broth

Strain	Parent strain			Colonial morphotype (serotype) of progeny[a] after in vitro culture for:		
	Source	Morphology	Serotype	30 days	60 days	90 days
C395	CF patient	Classic	1	Classic (1)	Classic (1) Enterobacter (1) Classic (PT)	
C632	CF patient	Classic	5	Mucoid (5)	Mucoid (5) Classic (NT)	
ENV105	Sink drain	Enterobacter	9	Mucoid (9) Classic (9) Dwarf (3)	Dwarf (9) Classic (PT) Enterobacter (PT)	
ENV115	Sink drain	Enterobacter	6	Classic (6) Enterobacter (PT)	Enterobacter (PT)	
ENV23	Sink drain	Classic	11	Classic (11)	Enterobacter (11) Rough (11)	Classic (11) Enterobacter (11) Dwarf (11) Rough (AA)
ENV10	Onion	Classic	11	Classic (11) Dwarf (11)	Classic (11) Dwarf (PT) Enterobacter (11) Mucoid (PT) Mucoid (11)	
ENV2	Lettuce	Classic	8	Classic (8) Mucoid (8) Enterobacter (8)	Classic (8) Dwarf (PT)	Classic (8) Mucoid (8) Dwarf (8) Enterobacter (8)
V106	Blood	Enterobacter	11	Classic (11) Dwarf (11)	Enterobacter (11) Mucoid (11)	
V22	Urine	Classic	9	Mucoid (PT) Enterobacter (PT) Dwarf (PT)	Classic (9) Enterobacter (PT)	
V76	Blood	Classic	6	Classic (6) Dwarf (10) Dwarf (PT)	Classic (6) Dwarf (PT) Dwarf (6)	

[a] PT, Polytypable; NT, nontypable; AA, auto-agglutinating.

charide with subsequent nonspecific agglutination by an antiserum. Emergence of nontypable or polytypable isolates was observed at similar frequencies in the strains from CF patients (9 [52.9%] of 17), other patients (29 [49.1%] of 57), or the environment (19 [57.6%] of 33).

LPS analysis. Outer membranes were purified, and LPS was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to determine whether the loss of typability was due to change in LPS from smooth to rough. LPSs from six montypable strains (three environmental and three from patients without CF) and their polytypable progeny are shown in Fig. 3. In each case, the monotypable parent strain (lanes 2, 4, 6, 10, 12, and 14) had the characteristic ladder of the O polysaccharide, as did the standard smooth laboratory strain (lane 8). The progeny derived after in vitro cultivation (lanes 1, 3, 5, 9, 11, and 13) lacked the O polysaccharide, as did the standard rough laboratory strain (lane 7). There were also differences in the core region (shown at the bottom of each lane), in which the parent strains had a higher-molecular-weight band that was lacking in the progeny. In addition the rough cores of the progeny migrated slightly more rapidly than the rough cores of the corresponding parent strains.

Serum sensitivity. Resistance to the bactericidal effect of normal human serum is dependent upon the presence of smooth LPS in *P. aeruginosa*. We therefore wished to determine whether the loss of typability after prolonged cultivation in acetamide broth was correlated with acquisition of serum sensitivity. All 20 monotypable parent strains (11 from patients without CF and 9 from the environment) were resistant to the bactericidal effect of 10% pooled normal human serum (Table 3). Polytypable strains were derived from each of these strains; 19 were more serum sensitive than their parents. Some of the progeny were resistant to 10% serum, but when tested with 25% serum all except for the polytypable progeny of strain V110 (which remained serum resistant) were more susceptible than their parents to the bactericidal effect of human serum. All 13 monotypable progeny strains were serum resistant.

Analysis of genetic relatedness of parent and progeny strains by using multilocus enzyme electrophoresis. Multilocus enzyme electrophoresis was performed on all strains in which emergence of the mucoid phenotype and/or rough LPS was observed. In all cases, the electrophoretic type of parent and progeny pairs were identical; this indicated that they were genetically related and that the latter were not contaminants.

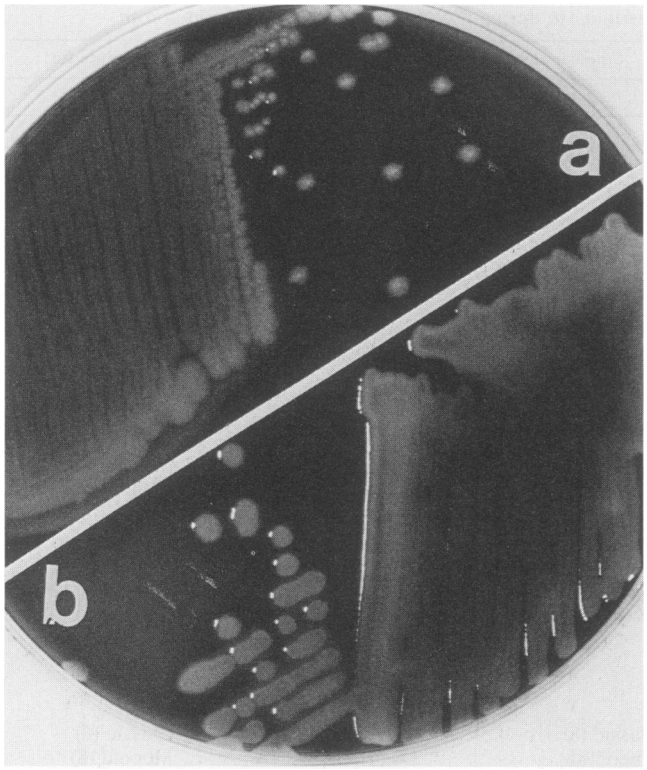

FIG. 2. A nonmucoid strain of *P. aeruginosa* (ENV48) isolated from river water (A) and the mucoid progeny (B) derived from it after 30 days of cultivation in acetamide broth. Note that the nonmucoid colonies in panel A have the characteristic feathered edges of the classic phenotype. The isolate in panel B has the slimy, glistening appearance with coalescence of adjacent colonies typical of the mucoid morphotype.

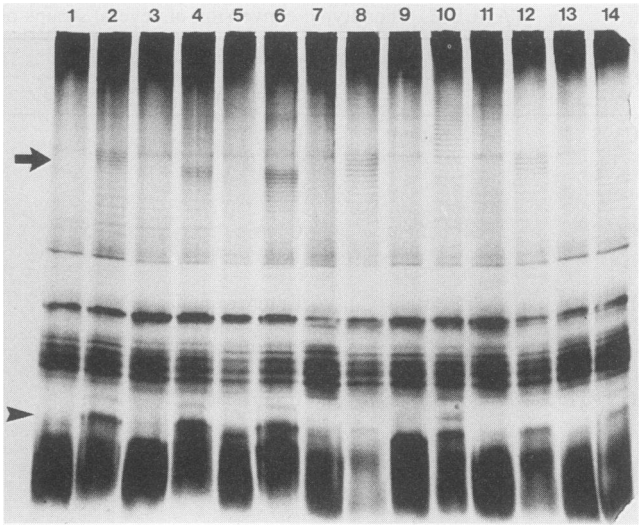

FIG. 3. Silver-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis of outer membranes from 14 isolates of *P. aeruginosa*. Lane 7 is from strain P1NM, a standard rough-LPS laboratory isolate (38). Lane 8 is from strain C655M, a standard smooth-LPS laboratory isolate. Lanes 2, 4, 6, 10, 12 and 14 are outer membranes from monotypable parent strains V81, V76, V75, ENV10, ENV8, and ENV2, respectively. The progeny isolates derived from the smooth-LPS strains are in the odd-numbered lanes (lanes 1, 3, 5, 9, 11, and 13) immediately preceding the parent strain. Note that the isolates in the odd-numbered lanes lack the well-developed O-polysaccharide ladder that is seen in the even-numbered lanes at the top half of the gel (arrow). The parent strains in the even-numbered lanes also have a higher-molecular-weight band in the core region, which is lacking in the progeny in the odd-numbered lanes (arrowhead).

Evaluation of mucoid strains. The mucoid material from mucoid strain A147, derived from ENV59 after cultivation for 30 days in acetamide broth, was analyzed. This strain was quite stable; it and nine other progeny strains remained mucoid after 20 in vitro passages on Columbia blood agar. The other mucoid in vitro convertants had variable stability of the mucoid phenotype with reversion to nonmucoid after 1 to 15 in vitro passages. The mucoid material was extracted from strain A147. It formed a flocculent white precipitate in ethanol, as does the mucoid exopolysaccharide from mucoid CF strains of *P. aeruginosa*. All three polysaccharides were composed exclusively of uronic acids; this suggested that the mucoid material from strain A147 was virtually the same as the mucoid exopolysaccharide of CF strains. These data suggested that the mucoid in vitro convertants were highly similar to mucoid strains from patients with CF in terms of their appearance and phenotypic stability and the chemical nature of the mucoid material they elaborated.

DISCUSSION

These investigations were designed to determine why peculiar mucoid, rough-LPS strains of *P. aeruginosa* colonize the respiratory secretions of patients with CF. Data from our studies (31) and from other laboratories (2) support the hypothesis that this bacterial phenotype evolves within the CF respiratory tract. These peculiar organisms appear to be derived from initial colonizing strains that are probably classical (i.e., nonmucoid with smooth LPS). It is critically important to determine the origin of these strains before establishing strategies for prevention of colonization and infection.

The factors within the CF respiratory tract which favor the conversion of *P. aeruginosa* to the mucoid phenotype have not been determined. It has been suggested that antimicrobial pressure selects mucoid variants (11); this is unlikely, since mucoid strains are not uniformly more resistant to antibiotics than their nonmucoid isogenic variants (6, 25). Furthermore, patients with CF are sometimes colonized with mucoid *P. aeruginosa* on first presentation for medical care, before ever receiving antibiotic therapy. In vitro conversion of *P. aeruginosa* from nonmucoid to the mucoid phenotype previously has been shown to occur under the influence of carbenicillin (11) or phages (26, 28). We are currently exploring the possibility that the phenotypic changes produced by our in vitro system are due to the effects of bacteriophages.

Our observations suggest that *P. aeruginosa* may convert to the mucoid, rough-LPS phenotype under environmental pressures present in the CF bronchopulmonary tree. Colonization and infection in these patients occurs in the endobronchial spaces, with minimal invasion of lung tissue. The bacteria may persist in this location for prolonged periods of time in coexistence with the host. Under these conditions *P. aeruginosa* may not have access to the nutrients usually available to bacteria causing invasive infection. A chronic low-grade infection is therefore established in which the bacteria must survive under conditions of nutritional limitation. We speculate that these conditions may select for

TABLE 3. Effect of prolonged culture of *P. aeruginosa* in acetamide broth on susceptibility to the bactericidal effect of human serum

Strain	Parent strain		Progeny		
			Monotypable		Polytypable: decrease in viability[a] (log_{10})
	Serotype	Decrease in viability[a] (log_{10})	Serotype	Decrease in viability[a] (log_{10})	
V75	5	0	5	0	>2
V76	6	0	6	0	1.04
V81	10	0			>2
V73	5	0	5	0	1.26
V93	3	0.24	3	0	1.23
V64	4	0	4	0	0.12
V5	1	0 (0)			0 (1.22)
V132	3	0	3	0	>2
ENV2	8	0	8	0	0.82
ENV8	1	0 (0)			0.24 (>2)
ENV10	11	0 (0)	11	0	0 (>2)
ENV5	1	0	1	0	>2
ENV43	6	0	6	0	>2
ENV46	1	0	1	0	0.44
V22	9	0 (0.14)	9	0	0.15 (1.83)
ENV105	9	0	9	0	0.52
ENV115	6	0 (0)			0.09 (>2)
V110	6	0 (0)			0 (0)
ENV47	9	0.03			>2
V41	9	0.15			>2

[a] Numbers represent decreases in viability in 10% pooled normal human serum. Numbers within parentheses are decreases in viability in 25% serum.

phenotypic variants of *P. aeruginosa* that are specially suited to survival in this ecological niche. It appears that not all strains of *P. aeruginosa* can convert to the mucoid, rough-LPS phenotype. Under our experimental conditions, only 19% became mucoid and 52% expressed rough LPS. Perhaps those strains capable of this conversion are better suited for survival in the CF bronchopulmonary secretions.

Mucoid *P. aeruginosa* are also found in patients with chronic bronchopulmonary disease (33) or chronic urinary tract disease (8), two other conditions in which the bacteria may survive for prolonged periods despite nutrient limitation. Mucoid variants of other gram-negative bacteria can also be recovered from the respiratory secretions of patients with CF (19, 24). This suggests that the peculiar environment of the CF bronchopulmonary space, rather than factors unique to *P. aeruginosa*, stimulates this phenotypic expression. Once mucoid variants arise, they may have a further survival advantage by virtue of their resistance to phagocytosis (1, 4).

The effects of nutrient deprivation on the expression of bacterial phenotypic characteristics have been studied extensively in various species (13), including a marine *Pseudomonas* species (43). Various phenotypic changes have been observed, including the elaboration and release of an exopolysaccharide (43), a process which may alter bacterial surface hydrophobicity and influence adhesion to other surfaces. In other studies, strains of *Vibrio cholerae* were found to degrade the O polysaccharide of LPS during periods of starvation, thereby becoming phenotypically rough (17). The polysaccharide is thought to serve as a source of nutrition for those bacteria. Both of these changes may by analogous to the acquisition of the mucoid, rough-LPS phenotype that we observed when culturing *P. aeruginosa* under conditions of nutrient limitation.

Although the phenotypic changes we observed may be due to the actions of bacteriophages (23, 26, 28), we feel that they could result from another type of genetic regulation by environmental conditions (27). Exogenous factors and signals can have profound effects upon the expression of various virulence factors of pathogenic bacteria by virtue of effects on gene transcription. For example, the concentration of environmental iron influences the expression of exotoxin A, elastase, and alkaline protease in *P. aeruginosa* (3, 36). Phenotypic modulation may permit bacteria to survive in diverse environments such as on inanimate surfaces or within animal hosts. The gene or genes that code for the elaboration of *P. aeruginosa* mucoid exopolysaccharide are under the control of another genetic element that can operate in the on or off mode (10). The factor(s) determining whether this switch is on or off is understood poorly. However, DeVault et al. recently observed that the transcriptional activation of genes for *P. aeruginosa* alginate production can be influenced by environmental factors (7). In future investigations, we will examine whether limitation of specific nutrients influences the transcription of the genes for mucoid exopolysaccharide production.

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LITERATURE CITED

1. **Baltimore, R. S., and M. Mitchell.** 1980. Immunologic investigations of mucoid strains of *Pseudomonas aeruginosa*: comparison of susceptibility to opsonic antibody in mucoid and nonmucoid strains. J. Infect. Dis. **141**:238–247.
2. **Bergan, T., and N. Høiby.** 1975. Epidemiological markers for *Pseudomonas aeruginosa*. Acta Pathol. Microbiol. Scand. Sect. B **83**:553–560.
3. **Bjorn, M. J., P. A. Sokol, and B.H. Iglewski.** 1979. Influence of iron on yields of extracellular products in *Pseudomonas aeruginosa*. J. Bacteriol. **138**:193.
4. **Cabral, D. A., B. A. Loh, and D. P. Speert.** 1987. Mucoid *Pseudomonas aeruginosa* resists nonopsonic phagocytosis by human neutrophils and macrophages. Pediatr. Res. **22**:429–431.
5. **Campbell, M. E., S. W. Farmer, and D. P. Speert.** 1988. New selective medium for *Pseudomonas aeruginosa* with phenanthroline and 9-chloro-9-[4-(diethylamino)phenyl]-9,10-dihydro-10-phenylacridine hydrochloride (C-390). J. Clin. Microbiol. **26**:1910–1912.
6. **Demko, C. A., and M. J. Thomassen.** 1980. Effect of mucoid property on antibiotic susceptibility of *Pseudomonas aeruginosa*. Curr. Microbiol. **4**:69–73.
7. **DeVault, J. D., A. Berry, T. K. Misra, A. Darzins, and A. M. Chakrabarty.** 1989. Environmental sensory signals and microbial pathogenesis: *Pseudomonas aeruginosa* infection in cystic fibrosis. Biotechnology **7**:352–357.
8. **Doggett, R. G., G. M. Harrison, and R. E. Carter, Jr.** 1971. Mucoid *Pseudomonas aeruginosa* in patients with chronic illness. Lancet **i**:236–237.
9. **Evans, L. R., and A. Linker.** 1973. Production and characterization of the slime polysaccharide of *Pseudomonas aeruginosa*. J. Bacteriol. **116**:915–924.
10. **Flynn, J. L., and D. E. Ohman.** 1988. Cloning of genes from mucoid *Pseudomonas aeruginosa* which control spontaneous conversion to the alginate production phenotype. J. Bacteriol. **170**:1452–1460.
11. **Govan, J. R. W., and J. A. M. Fyfe.** 1978. Mucoid *Pseudomonas aeruginosa* and cystic fibrosis: resistance of the mucoid form to

carbenicillin, flucloxacillin and tobramycin and the isolation of mucoid variants *in vitro*. J. Antimicrob. Chemother. **4**:233–240.
12. **Hancock, R. E. W., L. M. Mutharia, L. Chan, R. P. Darveau, D. P. Speert, and Pier GB.** 1983. *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis: a class of serum-sensitive nontypable strains deficient in lipopolysaccharide O side chains. Infect. Immun. **42**:170–177.
13. **Harder, W., and L. Dijkhuizen.** 1983. Physiological responses to nutrient limitation. Annu. Rev. Microbiol. **37**:1–23.
14. **Hitchcock, P. J., and T. M. Brown.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J. Bacteriol. **154**:269–277.
15. **Høiby, N., and S. Olling.** 1977. *Pseudomonas aeruginosa* infection in cystic fibrosis. Acta Pathol. Microbiol. Scand. Sect. C **85**:107–114.
16. **Høiby, N., and K. Rosendal.** 1980. Epidemiology of *Pseudomonas aeruginosa* infection in patients treated at a cystic fibrosis centre. Acta Pathol. Microbiol. Scand. Sect. B **88**:125–131.
17. **Hood, M. A., J. B. Guckert, D. C. White, and F. Deck.** 1986. Effect of nutrient deprivation on lipid, carbohydrate, DNA, RNA, and protein levels in *Vibrio cholerae*. Appl. Environ. Microbiol. **52**:788–793.
18. **Kelly, N. M., F. R. Falkiner, and C. T. Keane.** 1983. Acetamide broth for isolation of *Pseudomonas aeruginosa* from patients with cystic fibrosis. J. Clin. Microbiol. **17**:159.
19. **Kelly, N. M., F. R. Falkiner, C. T. Keane, M. X. Fitzgerald, and E. Tempany.** 1983. Mucoid gram-negative bacilli in cystic fibrosis. Lancet **i**:705.
20. **Knutson, C. A., and A. Jeanes.** 1968. A new modification of the carbazole analysis: application to heteropolysaccharides. Anal. Biochem. **24**:470–481.
21. **Kodama, H., M. Ishimoto, and A. Jono.** 1974. Isolation and serotyping of *Pseudomonas aeruginosa* from river water. J. Infect. Dis. Jpn. **48**:385–393.
22. **Levin, M. H., R. A. Weinstein, C. Nathan, R. K. Selander, H. Ochman, and S. A. Kabins.** 1984. Association of infection caused by *Pseudomonas aeruginosa* serotype O11 with intravenous abuse of pentazocine mixed with tripelenamine. J. Clin. Microbiol. **20**:758–762.
23. **Liu, P. V.** 1969. Changes in somatic antigens of *Pseudomonas aeruginosa* induced by bacteriophages. J. Infect. Dis. **119**: 237–246.
24. **Macone, A. B., G. B. Pier, J. E. Pennington, W. J. Matthews, Jr., and D. A. Goldmann.** 1981. Mucoid *Escherichia coli* in cystic fibrosis. N. Engl. J. Med. **304**:1445–1449.
25. **Markowitz, S. M., F. L. Macrina, and P. V. Phibbs, Jr.** 1980. Antimicrobial susceptibility of mucoid *Pseudomonas aeruginosa* and their spontaneously occurring non-mucoid derivatives. J. Antimicrob. Chemother. **6**:251–260.
26. **Martin, D. R.** 1973. Mucoid variation in *Pseudomonas aeruginosa* induced by the action of phage. J. Med. Microbiol. **6**:111–118.
27. **Miller, J. R., J. J. Mekalonas, and S. Falkow.** 1989. Coordinate regulation and sensory transduction in the control of bacterial virulence. Science **243**:916–922.
28. **Miller, R. V., and V. J. R. Rubero.** 1984. Mucoid conversion by phages of *Pseudomonas aeruginosa* strains from patients with cystic fibrosis. J. Clin. Microbiol. **19**:717–719.
29. **Muschel, L. H., L. A. Ahl, and M. W. Fisher.** 1969. Sensitivity of *Pseudomonas aeruginosa* to normal serum and to polymyxin. J. Bacteriol. **98**:453–457.
30. **Pedersen, S. S., C. Koch, N. Høiby, and K. Rosendal.** 1986. An epidemic spread of multiresistant *Pseudomonas aeruginosa* in a cystic fibrosis centre. J. Antimicrob. Chemother. **17**:505–516.
31. **Pier, G. B., D. DesJardins, T. Aguilar, M. Barnard, and D. P. Speert.** 1986. Polysaccharide surface antigens expressed by nonmucoid isolates of *Pseudomonas aeruginosa* from cystic fibrosis patients. J. Clin. Microbiol. **24**:189–196.
32. **Pitt, T. L., J. MacDougall, A. R. L. Penketh, and E. M. Cooke.** 1986. Polyagglutinating and non-typable strains of *Pseudomonas aeruginosa* in cystic fibrosis. J. Med. Microbiol. **21**:179–186.
33. **Rivera, M., and M. B. Nicotra.** 1982. *Pseudomonas aeruginosa* mucoid strain—its significance in adult chest diseases. Am. Rev. Respir. Dis. **126**:833–836.
34. **Schiller, N. L., and R. A. Hatch.** 1983. The serum sensitivity, colonial morphology, serogroup specificity, and outer membrane protein of *Pseudomonas aeruginosa* strains isolated from several clinical sites. Diagn. Microbiol. Infect. Dis. **1**:145–157.
35. **Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour, and T. S. Whittam.** 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl. Environ. Microbiol. **51**:873–884.
36. **Sokol, P. A., C. D. Cox, and B. H. Iglewski** 1982. *Pseudomonas aeruginosa* mutants altered in their sensitivity to the effect of iron on toxin A or elastase yields. J. Bacteriol. **151**:783.
37. **Speert, D. P., and M. E. Campbell.** 1987. Hospital epidemiology of *Pseudomonas aeruginosa* from patients with cystic fibrosis. J. Hosp. Infect. **9**:11–21.
38. **Speert, D. P., F. Eftekhar, and M. L. Puterman.** 1984. Nonopsonic phagocytosis of *Pseudomonas aeruginosa* strains from cystic fibrosis patients. Infect. Immun. **43**:1006–1011.
39. **Speert, D. P., D. Lawton, and S. Damm.** 1982. Communicability of *Pseudomonas aeruginosa* in a cystic fibrosis summer camp. J. Pediatr. **101**:227–229.
40. **Thomassen, M. J., and C. A. Demko.** 1981. Serum bactericidal effect on *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. Infect. Immun. **33**:512–518.
41. **Thomassen, M. J., C. A. Demko, C. F. Doershuk, and J. M. Root.** 1985. *Pseudomonas aeruginosa* isolates: comparisons of isolates from campers and from sibling pairs with cystic fibrosis. Pediatr. Pulmonol. 1:40–45.
42. **Wahba, A. H., and J. H. Darrell.** 1965. The identification of atypical strains of *Pseudomonas aeruginosa*. J. Gen. Microbiol. **38**:329–342.
43. **Wrangstadh, M., P. L. Conway, and S. Kjelleberg.** 1986. The production and release of an extracellular polysaccharide during starvation of a marine *Pseudomonas sp.* and the effect thereof on adhesion. Arch. Microbiol. **145**:220–227.
44. **Zierdt, C. H., and P. J. Schmidt.** 1964. Dissociation in *Pseudomonas aeruginosa*. J. Bacteriol. **87**:1003–1010.
45. **Zimakoff, J., N. Høiby, K. Rosendal, and J. P. Guilbert.** 1983. Epidemiology of *Pseudomonas aeruginosa* infection and the role of contamination of the environment in a cystic fibrosis clinic. J. Hosp. Infect. **4**:31–40.