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Mucoid Conversion by Phages of *Pseudomonas aeruginosa* Strains from Patients with Cystic Fibrosis

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A total of 21 of 22 independent isolates of cystic fibrosis-associated *Pseudomonas aeruginosa* were found to be lysogenic for DNA-containing, complex capsid viruses. Several of the phages demonstrated the ability to select mucoid cells from populations of nonmucoid bacteria. Conversion to mucoid growth was more frequently achieved when phages were isolated from mucoid as opposed to nonmucoid cystic fibrosis-associated strains.

In progressive *Pseudomonas aeruginosa* infections of the lungs of cystic fibrosis (CF) patients, strains which produce copious amounts of alginate (mucoid variants) are isolated from more than 90% of affected individuals (2, 3). Infection by temperate bacteriophages has been proposed as a mechanism for the conversion of *P. aeruginosa* from the nonmucoid to the mucoid phenotype (4, 13, 20). *P. aeruginosa* bacteriophages are present in the sputa of patients with chronic *P. aeruginosa* bronchopulmonary infections (19) and, therefore, potentially influence the *P. aeruginosa* population at these sites of infection. Martin (14) observed that mucoid clones could be subcultured from the bacterial lawn surrounding areas of phage lysis. Zierdt and Schmidt (21) correlated dissociation of mucoid isolates from CF patients with changes in their phage-typing characteristics and suggested that the mucoid phenotype was the result of a phage-mediated conversion. Govan (6) has reported "conversion" of nonmucoid strains by selection for survivors of phage E79 infection. We examined the lysogenic state of strains of *P. aeruginosa* isolated from the sputa of CF patients to determine whether viruses potentially capable of inducing phage conversion are present in these isolates.

Twenty-two strains of CF-associated *P. aeruginosa* (Table 1) were tested for the presence of temperate bacteriophages. Overnight cultures of each CF-associated isolate were grown in Luria broth medium (15) and centrifuged (5,000 to 7,000 rpm for 10 min) to remove bacterial cells and cellular debris. The supernatant fluid was then filtered through a membrane filter (0.45 μm mean pore size; Millipore Corp.) and tested for the presence of PFUs by using strain PAO1 (17), strain PAT2 (20), and each of the other CF isolates as hosts. Of the 22 strains tested, only RM1414 did not release phage detectable as plaques on at least one of the tester strains (Table 1). Titers ranging from 10^6 to 10^9 PFU/ml were obtained. All plaques were of a typical turbid morphology indicative of temperate bacteriophages. A total of 8 strains produced higher titers of phage when grown at 37°C; the other 11 strains produced higher titers of spontaneously induced phage when grown at 30°C (Table 1). Although cleared lysates of RM1414 did not produce plaques, they did have the ability to kill almost all of the strains when cross-streaked against the various strains. RM1414 may contain a narrow host range or defective phage or a strain-specific pyocin. Since studies have shown that most *P. aeruginosa* isolates are lysogenic and often multilysogenic (8), it is likely that our indicator set was too short to identify a strain which was permissive for plaque formation by phage from this strain.

Several criteria were chosen to characterize the phages isolated from these CF isolates. First, radioactively labeled ([^{3}H]adenine) nucleic acid samples isolated from purified virions of the various phages by the method of Miller et al. (16) were tested for sensitivity to DNase I (12) and RNase A (13). In all cases, the nucleic acid was found to be sensitive to DNase I and insensitive to RNase A. Thus, it can be concluded that the genomes of these phages are composed of DNA. Second, electron microscopic examination (16) revealed complex capsids with elongated heads and flexible tails. Some variation in the dimensions of the heads and tails was noted in phages isolated from different CF-associated bacterial strains, but no striking differences in capsid morphology were observed. Third, sensitivity to inactivation of the virion by 5% ether or 5% chloroform was tested. The majority of the phage isolates were resistant to both chemicals, but isolates sensitive (i.e., reduction in titer of >99% in 4 days) to ether (φ1404), chloroform (φ1409 and φ1417), or both (φ1402) were also detected. Fourth, bacteriophages from the various CF isolates were examined to determine whether they were members of any of the recognized serogroups of *P. aeruginosa* phages (7, 9, 11). Neutralizing antisera were prepared against representative phages of each serological group which contains temperate phages (9). These included phages B3, D3, F116, and G101 (7, 9, 11, 18). These antisera were used in neutralization tests against each of the phages isolated from CF-associated *P. aeruginosa* strains (9). No cross-neutralization was found with any of the CF phages, except that φ1417 was neutralized by anti-phage G101 serum. Therefore, it would appear that the majority of these phages are not members of a recognized serogroup of *P. aeruginosa* temperate viruses.

The data from this study indicate that CF-associated isolates of *P. aeruginosa* are usually, if not always, lysogenic for one or more temperate phages. The phages found in CF sputa by Tejedor et al. (19) may have their source in the spontaneous induction of lysogenic bacteria found in the bronchopulmonary tract of these patients.

Martin (14) and Govan (6) have reported that certain bacteriophages are capable of converting nonmucoid strains to the mucoid phenotype. In their studies, neither the phages nor the bacteria were isolated from CF-associated *P. aeru-*

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TABLE 1. Plaquing capability of bacteriophage isolated from CF-associated isolates

Strain[a]	Plaquing characteristics	
	Optimum temp[b] (°C)	Host strains[c]
Mucoid strains[d]		
RM1399	30	PAO1, PAT2, RM1404, RM1406
RM1400	30	RM1404, RM1406, RM1417, RM1418
RM1402	30	PAO1, PAT2, RM1404, RM1405, RM1410
RM1404	30	RM1399, RM1400, RM1406, RM1407, RM1409, RM1410, RM1411, RM1414, RM1418
RM1406	37	PAO1, PAT2, RM1404, RM1405, RM1407, RM1411
RM1407	37	PAO1, PAT2, RM1402, RM1406, RM1408
RM1412	37	PAO1, PAT2
RM1414[e]		
RM1415	30	PAO1, RM1404, RM1406, RM1407, RM1410, RM1411, RM1412
RM1417	30	PAT2, RM1399, RM1404, RM1407, RM1412, RM1418
RM1418	37	PAO1, RM1406
RM1420	30	PAO1, PAT2
Nonmucoid strains[d]		
RM1401	30	RM1400, RM1404, RM1410
RM1403	30	PAO1, PAT2, RM1401, RM1409, RM1410, RM1411
RM1405	37	PAO1, PAT2, RM1401, RM1403, RM1404, RM1406, RM1407, RM1408, RM1410, RM1411, RM1413
RM1408	30	RM1404, RM1406, RM1407, RM1411, RM1413
RM1409	30	RM1401, RM1404, RM1407, RM1408, RM1410, RM1411, RM1412, RM1413
RM1410	30	RM1404, RM1408
RM1411	37	PAO1, PAT2, RM1406, RM1410
RM1413	37	PAO1, PAT2, RM1404, RM1411
RM1416	30	PAO1, PAT2, RM1404, RM1406, RM1407, RM1412, RM1415
RM1419	37	PAO1

[a] Strains RM1399 through RM1403 were from W. Dow, Vanderbilt University; RM1404 through RM1413 were from C. H. Zierdt, National Institutes of Health; RM1414 through RM1419 were from A. von Graevenitz, Yale University; and RM1420 was from the Centers for Disease Control, Atlanta.

[b] Optimal titers of spontaneously induced phage were produced when cultures were grown at this temperature. Plating efficiencies on the various indicator strains were also maximal at this temperature.

[c] Cleared lysates of the strain produced plaques on these strains.

[d] Colony morphology when grown on Luria agar (15).

[e] Cleared lysates of RM1414 failed to produce plaques on any of the strains tested. These lysates were able to induce killing of all strains except RM1404 and RM1414.

ginosa infections of the lung. We wished to test the bacteriophages which we had identified for their ability to convert bacteria to the mucoid phenotype. Cross streaks of nonmucoid bacteria (either laboratory strains or strains isolated from CF infections) were made against bacteriophages (10^9 PFU/ml) isolated from CF-associated strains. After 24 h of incubation, the plates were observed for changes in colony morphology at the bacteria-phage intersection. When mucoid colonies were observed, they were picked, and the mucoid phenotype of the isolated colonies was confirmed by subcloning.

Phage-mediated conversion could be demonstrated with 6 of the 22 phage lysates (Table 2). Two of these phages were from nonmucoid CF strains. One of the six strains which gave rise to converting lysates (RM1414) was the only strain from which we were unable to obtain PFUs. The converting factor found in this strain was removed by filtration of cell-free lysates through a Millipore filter with a mean pore size of 0.22 μm. Filtration of cell-free lysates from this strain through 0.45-μm filters did not remove the factor. Of the 10 CF-associated nonmucoid strains studied, only RM1410 and RM1411 could be converted to mucoid growth by these bacteriophages. These phages were also unable to convert the laboratory strains PAO and PAT. Conversion of strain PAO to mucoid growth was achieved with two well-characterized phages, F116 and E79. Conversion of non-PAO strains by E79, a virulent bacteriophage (7), has been reported by Govan (6).

Phage mediation of induction of alginate synthesis is consistent with these results. This induction could be due to one of two mechanisms. The mucoid phenotype could be a result of lysogenic conversion in which the introduction of phage-encoded genes into the cell is responsible for the alteration in the phenotype of the host (9). This hypothesis is supported by the fact that the vast majority of conversions were achieved by phages isolated from mucoid as opposed to nonmucoid strains of *P. aeruginosa*. Equally plausible is that the phages may act as selective agents for a subpopulation of mucoid cells. Here, one might postulate that the mucoid layer acts to inhibit phage infection, thereby increasing the survival chance of the mucoid cell over that of the nonmucoid cell. This phenomenon is known to occur with *Escherichia coli* and phage lambda (5). This hypothesis is supported by several observations from this and former studies. Conversion by phages isolated from nonmucoid strains, although less frequent, was observed. We have found, as did Govan (6), that conversion can be accomplished by the virulent phage E79 which cannot establish lysogeny in PAO hosts. The induction of the mucoid phenotype by antibiotics (6) and pyocins (10) has been reported. Martin (14) failed to identify lysogens among the phage-induced, mucoid variants which she isolated in her study. In addition, Fyfe and Govan (4) and Banerjee et al. (1) have identified chromosomal loci involved in the biosynthesis and control of alginate production. the accumulation of data requires that the hypothesis

TABLE 2. Phage-mediated conversion of nonmucoid strains to the mucoid phenotype

Phage[a]	Strain converted[b]	
	RM1410	RM1411
ϕ1403	+	+
ϕ1404	+	+
ϕ1406	−	+
ϕ1409	−	+
ϕ1414	+	+
ϕ1415	+	+

[a] Cleared lysates of these strains were able to convert the nonmucoid strains listed to mucoid growth.

[b] +, Conversion to mucoid growth; −, no change in colony morphology.

that CF strain bacteriophages give mucoid forms a selective advantage must be considered as a viable mechanism for mucoid conversion in *P. aeruginosa*.

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