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Basic Characterization of a *Pseudomonas aeruginosa* Pilus-Dependent Bacteriophage with a Long Noncontractile Tail

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Bacteriophage PO4 has been found to depend on the presence of pili for the infection of its host organism, *Pseudomonas aeruginosa*. Unlike other pilus phages, which either contain RNA and are "spherical" or contain single-stranded DNA and are filamentous, PO4 has a head and a long noncontractile tail. This paper describes its basic characters, and a quantitative study is made of its adsorption to exponential-phase cells of piliated and nonpiliated strains of *P. aeruginosa*. PO4 is found to contain double-stranded DNA and appears to be virulent towards its two host strains.

Bacteriophages which adsorb to the host and infect by using bacterial pili have hitherto been restricted to two basic morphological types, the so-called "spherical" RNA-containing phages and the filamentous bacterial viruses with single-stranded DNA. The more common tadpole-shaped phages with heads and tails adsorb to the host cell wall and occasionally to bacterial flagella. The exception is *Pseudomonas aeruginosa* bacteriophage PO4 (9), which has been found by electron microscopy to adsorb to the polar pili of its host. Preliminary observations (9) also showed that adsorption caused an increase in the number of pili present and that PO4 could not infect pilus-less host mutants. This paper provides more information on the nature of the virion and describes experiments which confirm the evidence regarding the use of pili as receptors.

MATERIALS AND METHODS

Bacteria and bacteriophages. *P. aeruginosa* strains PAO1 and PAO68 were kindly supplied by Bruce Holloway, Monash University, Clayton, Victoria, Australia. *P. aeruginosa* strain K (ATCC 25102) was streaked, and cultures from a single colony (laboratory clone number KSC) were used here. A full list of strains, together with explanatory details, is given in Table 1.

The three *P. aeruginosa* bacteriophages used were the RNA pilus phage PP7 (3), the filamentous pilus phage Pf (14; D. E. Bradley, Can. J. Microbiol., in press), ATCC 25102B, and phage PO4 (9).

Routine culture media and methods. Oxoid nutrient broth was used throughout for both liquid and 2% (wt/vol) agar plate cultures. Double-layer agar plates (1) for phage titration or lytic activity spot tests contained 0.3% (wt/vol) agar in the top layer. Bacteriophages other than Pf were grown by the confluent lysis of the host strain in 6-in (15.24 cm) diameter double-layer plates. Extraction of the phages was achieved by pouring about 7 ml of broth on to each plate. After gentle agitation for 3 h the liquid was decanted, and bacteria were removed by centrifugation. Titers of about 5×10^9 PFU/ml were obtained in this way. Phage Pf was prepared as described elsewhere (D. E. Bradley, Can. J. Microbiol., in press), by growing an infected broth culture of strain K with shaking at 37 C for 24 h. Bacteriophages were titrated by using standard methods (1), but there was a tendency for plaques to be obscured by bacterial overgrowth. Plates for each serial dilution were, therefore, prepared in triplicate. PO4 preparations grown on strains K and PAO1 were designated PO4·K and PO4·PAO1, respectively.

Growth curves for phage-infected bacteria were obtained by following the optical density of cultures shaking at 37 C. Optical density was converted to cells per milliliter by calibration against viable counts obtained from an uninfected culture.

Determination of the lytic activity and EOP of phage PO4 on strains of P. aeruginosa. The lytic activity of phages PO4, Pf, PP7, and PB1 against strains of *P. aeruginosa* was determined by spotting high-titer phage suspensions on to double-layer agar plates and noting the presence or absence of clearing after overnight incubation. Efficiency of plating (EOP) with reference to strain K was determined as follows. Suitable phage dilutions were plated on strain K and the strain under test. The EOP is expressed as the ratio of the titer obtained with the test strain to that obtained with K.

Preparation of phage-resistant mutants and tests for lysogeny. In the first of two methods, strain K or PAO1 was plated (double-layer agar plates) with

TABLE 1. *List of strains of Pseudomonas aeruginosa*[a]

Strain	Description	Isolation method or source
K	Host for phages PO4 and Pf	ATCC 25102, single colony reisolation
K3fla$^-$	Strain K with no flagella (Bradley, in press)	From culture containing 1% (wt/vol) sodium dodecyl sulphate (Bradley, in press)
K/PB1	Strain K resistant to contractile phage PB1 (10)	Spot test method
K/1PO4$^-$	Strain K resistant to phage PO4	From confluently lysed double-layer agar plate
K/2PO4$^-$	Strain K resistant to phage PO4	From infected broth culture
K3fla$^-$/PO4$^-$	K3fla$^-$ resistant to phage PO4	From confluently lysed double-layer agar plate
K/12Pf$^-$2	Strain K resistant to phage Pf (5; Bradley, in press)	Previously 25102/12Pf (5), spot test method
PAO1	Host for RNA phage PP7 (3), resistant to phage Pf (5)	ATCC 25247
PAO68	Derivative of PAO1 resistant to phage PP7 (5)	B. W. Holloway, Monash University, Australia
PAO1/PO4$^-$	PAO1 resistant to phage PO4	From confluently lysed double-layer agar plate
PAO1/PP7$^-$	PAO1 resistant to phage PP7 (5)	Previously 1/7 (5), spot test method

[a] A suffix$^-$ means that the strain is not lysogenic for, nor is it carrying the phage to which it is resistant.

an excess of phage PO4 (grown on the same strain). Surface colonies were streaked and tested for resistance to PO4, and lysogeny. The second procedure was to streak a broth culture which had been lysed by PO4. After incubation, single colonies were picked for testing. Each clone was streaked six times and screened for lysogeny in the following sequence starting with a bacterial spot test. Bacteria were spotted on double-layer agar plates of their respective strains of origin. After overnight incubation, a clear area surrounding the bacteria under test indicated the presence of phage and possible lysogeny. Next, these clones were grown for 24 h in the presence of anti-PO4 serum (prepared in rabbits by standard methods) at a concentration which inactivated 99.9% of a PO4 suspension in 10 min. The cells were then washed twice in broth by centrifugation to remove antibodies, and streaked. Bacteria from the resulting culture were inoculated into plain broth and grown for 4.5 h, when they were removed by two cycles of centrifugation at 7,000 $\times$ *g* for 10 min. The resulting cell-free fluids were tested for the presence of phage by pipetting 0.1-ml samples on to the surface of double-layer agar plates of the appropriate indicator strain. A small quantity of dilute PO4 was placed on another part of the same plate as a control. After the plate dried for 30 min uncovered and was incubated overnight, the presence or absence of phage in the sample was detectable by plaque formation. The sensitivity was estimated at 20 PFU/ml. The method was preferred to the phage assay procedure because it afforded a control, which produced discrete plaques, to validate negative results. The absence of plaques in the culture fluids indicated that the clone under test was nonlysogenic.

Adsorption of phage PO4 to strains of P. aeruginosa. Quantitative adsorption tests were carried out as follows. Bacteria were grown to about 2×10^9 cells/ml in shake culture. A 3-ml amount of this was mixed with 3 ml of phage suspension at 4×10^7 PFU/ml. In blank samples the bacterial culture was replaced with broth. The mixtures (including a blank) were shaken at 37 C for 20 min, and then bacteria were removed by two cycles of centrifugation at 7,000 $\times$ *g* for 10 min. Dilutions were plated (double-layer agar plates) with indicator strain, and the plaques were counted to give the number of phages remaining unadsorbed. The amount of phage adsorbed over 20 min was calculated as a percentage of the titer of the blank. The same phage suspension was used for a series of determinations, a blank being included with each. The titers obtained for the blanks were all in the range 1.9×10^7 to 2.1×10^7 PFU/ml.

A high multiplicity of infection (MOI) was needed for the electron microscopy of virions adsorbed to host cells. Exponential-phase bacteria were, therefore, diluted to between 2×10^8 and 6×10^8 cells/ml according to the MOI desired. The suspension (volume 1 to 2 ml) was mixed in a suitable ratio, with phage preparation at about 5×10^9 PFU/ml. After incubation with shaking for 10 min, bacteria were mounted for electron microscopy (see below).

Electron microscopy. Electron microscope specimens were prepared for phage morphological studies, pilus counts on bacteria, etc., using standard negative staining with 1% (wt/vol) sodium phosphotungstate solution. Strain K bacteria (with or without phage adsorbed) were mounted on grids for qualitative observation by centrifugation. The suspension was placed in centrifuge tubes with carbon-coated electron microscope specimen support grids on the bottom. After centrifugation in swing-out buckets at 1,000 $\times$ *g* for 3 min, grids were removed, washed, and negatively stained. For structural studies, phage PO4

was mounted by floating a carbon-coated grid on a suspension for about 10 min, followed by washing and negative staining.

Pili were counted on bacteria in the electron microscope after labeling with anti-pilus serum prepared by injecting a pilus suspension into rabbits (8). Bacteria were grown for 3.5 h (about 6×10^8 cells/ml) in shake culture, and a carbon-coated grid was immersed in the culture and moved rapidly perpendicular to the grid surface for about 1 min, causing bacteria to stick to the support film. The grid was then immersed in water for a few seconds, next in anti-pilus serum (1:1 in water) for 10 s, followed by two washes in water and negative staining. For *P. aeruginosa* strain PAO1 and its derivatives, which adhered to the carbon film more easily, a carbon-coated grid was floated on the culture for about 1 min followed by labeling with homologous anti-pilus serum, washing, and negative staining. The number of pili present was estimated by counts on 100 poles (*P. aeruginosa* pili are strictly polar) and is expressed as pili per pole. In the absence of pili, at least 100 cells (200 poles) were checked.

Fluorescent staining with acridine orange. The acridine orange fluorescent staining method for ascertaining the type and strandedness of the nucleic acid in a virus was carried out as described elsewhere (2). A PO4 suspension was treated with 10 μg of DNase (deoxyribonuclease I, Sigma Chemical Co.) per ml and 100 μg of RNase (ribonuclease A, Sigma Chemical Co.) per ml at 37 C for 1 h. The enzymes were removed by washing the virus by centrifugation. The virus was finally suspended in phosphate-buffered saline. Droplets were dried on to microscope slides, fixed in Carnoy fluid for 5 min, rinsed in alcohol, and dried in a stream of warm air. Slides were stained in 0.01% (wt/vol) acridine orange in citric acid plus phosphate buffer (0.1 M citric acid, 6.0 ml; 0.15 M Na_2HPO_4, 4.0 ml; pH 3.8) for 5 min. After soaking in 0.15 M Na_2HPO_4 for 15 min, slides were viewed under ultraviolet light (257 nm). Colors were noted, together with the changes produced by further treatment in molybdic or tartaric acids (2). The RNase and DNase sensitivity of the dried droplets was also tested (2). A suspension of coliphage T5 was treated simultaneously as a control.

RESULTS

Isolation and structure of phage PO4. The bacteriophage was isolated on strain K from untreated sewage from Ormiston, Scotland (9). The plaques were clear, 1 mm in diameter, and raised like blisters. It was found that the raised appearance was characteristic of the bacterium, not the virus, since phages PB1 (contractile) and Pf (filamentous) also formed raised plaques. PB1 and PO4 plaques were not raised with strain PAO1.

The general appearance of phage PO4 has already been described briefly (9) and is shown in Fig. 1. It belongs to the group with long noncontractile tails, having a head with a more or less hexagonal outline and a flexible tail with a small indistinct bar-shaped structure (in profile) near the tip (Fig. 1, arrow). Preparations of the virus contained numerous headless tails. Empty virions such as that shown in Fig. 2 often had an appearance consistent with an octahedron, a common shape among bacteriophages (4). A few headless tails (Fig. 3) showed a double-disk assembly where the head had been attached. A proportion of the virions had heads which were smaller than normal (Fig. 6), the tail being the usual length complete with bar-shaped structure. The same micrograph shows an empty virion with a normal head and hollow tail. Fine striations were visible on the tail (Figs. 4, 5). In short, phage PO4 is typical of its morphological group.

The various dimensions calibrated against 264-nm polystyrene latex spheres are as follows: side length of octahedral head, 58 nm; size of smaller head, 35 nm; tail length, 186 nm; tail thickness, about 10 nm; periodicity of tail striations, about 3.8 nm; width of tail bar, about 33 nm; thickness of tail bar, about 8 nm.

Nucelic acid type. The results of fluorescent staining and nuclease sensitivity tests are listed in Table 2. The colors obtained with PO4 are the same as with the 2-DNA coliphage T5 and correspond to 2-DNA standard colors (2). The isolated nucleic acid is also sensitive to DNase. Thus, as might be expected from its morphology, PO4 contains 2-DNA. It was noted that the presence of 100 μg of DNase per ml or 20 μg of RNase per ml in the top layer of double-layer agar plates did not inhibit plaque formation.

Tests for lysogenization by phage PO4. 33 PO4-resistant clones of strain K and 34 of strain PAO1 were isolated from PO4-infected plates and broth cultures; they were tested for PO4 production and hence possible lysogeny. Of these, 58 gave no phage, as indicated by the bacterial spot test. All of the remaining nine were "cured" by growth in the presence of anti-PO4 serum, their culture fluids having less than 20 PFU/ml when grown in serum-free broth. On this evidence the phage is considered virulent towards strains K and PAO1.

Because of their extensive use in experiments, the following strains were grown without anti-PO4 serum, and their culture fluids assayed for PO4 as a confirmation of the bacterial spot test: K/1PO4$^-$, K/2PO4$^-$, K3fla$^-$/PO4$^-$, and PAO1/PO4$^-$. All gave no detectable phage (<20 PFU/ml).

Host range and the adaptation of PO4 to growth on strain PAO1. Spot tests indicated that phage PO4 could grow on strain PAO1 as well as on strain K. It did not lyse one F$^+$ and

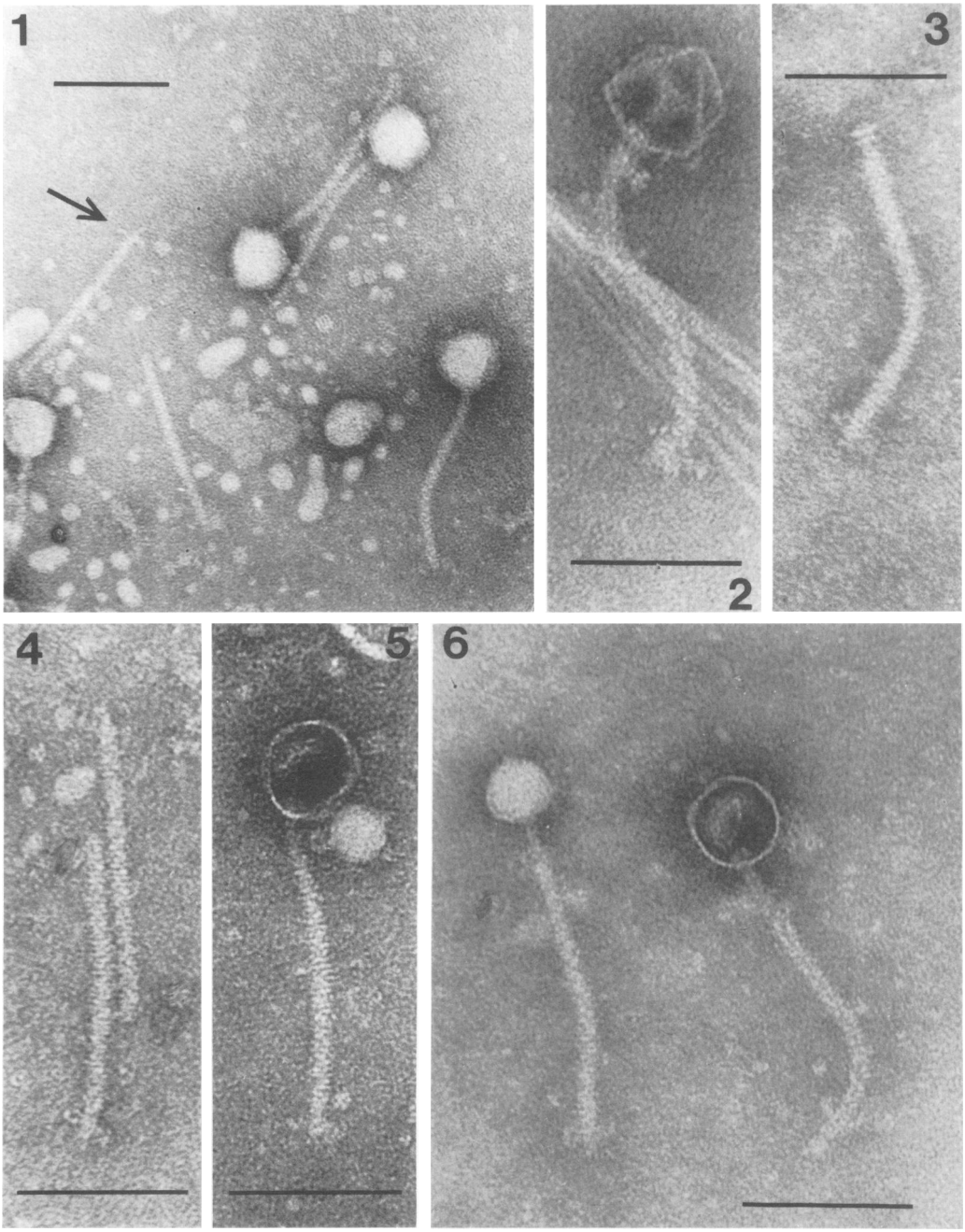

FIG. 1. *Negatively stained PO4 virions. Arrow marks bar-shaped structure at tail. Scale, 100 nm in Fig. 1 through 6.* ×*140,000.*

FIG. 2. *Empty PO4 virion showing octahedral shape of head.* ×*240,000.*

FIG. 3. *Headless tail showing double disk where head was attached.* ×*225,000.*

FIG. 4. *Headless tails showing striations.* ×*240,000.*

FIG. 5. *Empty virion with solid striated tail.* ×*240,000.*

FIG. 6. *Small-headed virion (full) and normal virion (empty).* ×*240,000.*

TABLE 2. *Colors obtained with the acridine orange staining of phage PO4*

Specimen	Nucleic acid	Color obtained after treatment with:				
		Na_2HPO_4	Molybdic acid	Tartaric acid	RNAse[a]	DNAse[a]
PO4	?	Bright green	Bright green	Reddish orange	−	+
T5	2-DNA	Bright green	Bright green	Reddish orange	−	+
Standard	2-DNA	Green	Green	Orange	−	+
Standard	2-RNA	Green	Green fading	Red	−	−
Standard	1-DNA	Red	Paler green	Paler green	−	+
Standard	1-RNA	Red	Paler red	Paler red	+	−

[a] Resistance to a nuclease, −; sensitivity, +.

two F^- strains of *Escherichia coli*. Its activity on strains K and PAO1 was further studied by measuring the EOP on PAO1 relative to K. Two preparations were tested: PO4·K (the phage grown on K) and PO4·PAO1 (PO4 adapted to growth on strain PAO1 by three cycles of plaque-picking followed by normal propagation on PAO1 plates). The results (averages of three determinations) are given in Table 3. It can be seen that a considerable increase in EOP has been achieved by the adaptation procedure, probably because of the selection of a more favorable clone of the phage.

Growth of PO4-infected bacteria. To ascertain the time available for phage adsorption tests prior to lysis, growth curves were determined for infected bacteria under the same conditions. The results (Fig. 7) permit comparison of the behavior of strains K and PAO1 after PO4-infection. The curve for strain K (MOI 1) shows one-step lysis about 1 h and 15 min after infection (arrow A); an increase in MOI did not alter this time significantly. No such lysis is shown with PAO1 even at an MOI of 10, although in both curves illustrated there is a slight temporary reduction in bacterial growth rate (arrows B and C) at 1 h and 27 min (MOI 10) and 1 h and 42 min (MOI 1). With the experiment at MOI 1, phage was added just as the culture was entering the exponential phase, but this did not increase the subsequent bacterial growth rate reduction (Fig. 7C) relative to the other PAO1 curve (Fig. 7B).

Piliation of various P. aeruginosa strains and their susceptibility to PO4 and other phages. Table 4 shows the relationship between piliation and sensitivity to certain bacteriophages as shown by the spot test. The pili were counted after labeling with homologous anti-pilus serum for 10 s to prevent possible pilus retraction and yet not to allow sufficient time for new pili to be formed under stimulation by antibody adsorption (7). These numbers represent what have been termed primary pili (7). The contractile phage PB1 (10) is active on all strains except the mutant which is resistant to it ($K/PB1^-$). The filamentous pilus phage Pf (14; D. E. Bradley, Can. J. Microbiol., in press) grows on piliated mutants of strain K only, and the RNA-containing pilus phage PP7 (3) is restricted in activity to strain PAO1. Strain PAO68, derived from PAO1, is also piliated but

TABLE 3. *Efficiency of plating of phage PO4 on P. aeruginosa strains K and PAO1*

Strain	Phage preparation	EOP[a]
K	PO4·K	1
PAO1	PO4·K	0.001
K	PO4·PAO1	1
PAO1	PO4·PAO1	0.24

[a] See Materials and Methods for definition.

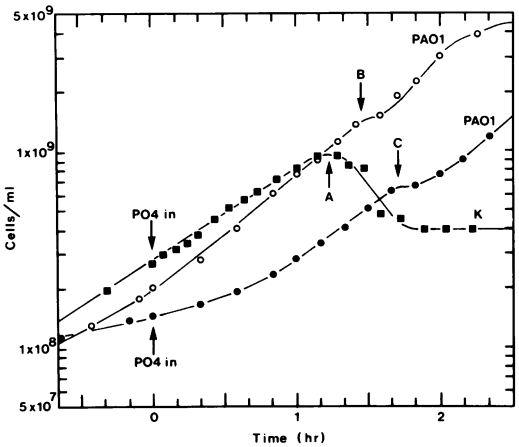

FIG. 7. *Growth curves for PO4-infected cultures. Strain PAO1:* (○) *MOI, 10;* (●) *MOI, 1. Strain K:* (■) *MOI, 1. Phage was introduced for all cultures at 0 h on the time scale. Arrow A marks onset of lysis for strain K. Arrows B and C show slight temporary reduction in growth rate for strain PAO1.*

TABLE 4. *Relationship of piliation and susceptibility to bacteriophages for mutants of P. aeruginosa strains K and PAO1*

Strain	Pili/pole	Poles piliated %	Lytic activity of phages			
			PO4	Pf	PP7	PB1
K	3.6	74	+	+	−	+
K3fla	4.4	74	+	+	−	+
K/PB1	0.72	33	+	+	−	−
K/1PO4⁻	0	0	−	−	−	+
K/2PO4⁻	0	0	−	−	−	+
K3fla⁻/PO4⁻	0	0	−	−	−	+
K/12Pf⁻2	0	0	−	−	−	+
PAO1	2.1	80	+	−	+	+
PAO68	3.2	87	−	−	−	+
PAO1/PO4⁻	0	0	−	−	−	+
PAO1/PP7⁻	0	0	−	−	−	+

is resistant to PP7 because its pili are unable to retract and permit RNA penetration (6, 8). Similarly, PAO68 is resistant to phage PO4, which lyses all other piliated strains including PAO1.

Adsorption of phage PO4 to P. aeruginosa strains. The adsorption of PO4 is shown in Table 5 for various mutants of *P. aeruginosa* under similar conditions. Phage suspensions grown on strain K (labeled PO4·K) and PAO1 (labeled PO4·PAO1) were used. The figures shown here are the percentage of phage adsorbed over 20 min with reference to a blank, the titer of which (2×10^7 PFU/ml) represented 0% adsorbed in each of a series of identical determinations. The MOI was 0.02 (50 bacteria per phage). Titers obtained to within ±2% of the blank are listed as giving <2% adsorption. Table 5 includes primary pili/pole from Table 4 so that adsorption and piliation may be correlated. It can be seen that only piliated strains adsorb phage PO4 significantly. Strain PAO1 and its derivative strain PAO68 are much less efficient than the sensitive K strains. There was a significant improvement in adsorption by PAO1 after the phage had been adapted to grow on it (preparation PO4·PAO1).

It has been shown elsewhere (D. E. Bradley, in press) that the filamentous pilus phage Pf does not adsorb to strain K/1PO4⁻. A similar result was obtained here with strain K/2PO4⁻ which adsorbed 3.5% of a Pf suspension over 20 min, compared with 97.6% for strain K (MOI 0.01, blank titer 6×10^6 PFU/ml).

Electron microscopy of PO4 adsorption. Electron micrographs of cells of *P. aeruginosa* strain K (5-h culture) with phage PO4 adsorbed at MOI 10, showed that the virions had a marked preference for the poles of the bacterium (Fig. 8, 11). In some cases, phage particles were also found attached to pili (Fig. 8, arrowed). They were not always close to the cell but also occurred near the pilus tip (Fig. 9). The phages were attached by the tips of their tails, and in Fig. 10 the bar-shaped structure appears to have hooked round the pilus filament. Like RNA phage PP7, the PO4 virions adsorbed along the length of the pilus rather than at the tip. Full, empty, and headless particles are attached. All the particles at the cell pole appear to be empty. It was also noted that there was a relationship between the number of pili and the number of adsorbed phage particles on each pole. In Fig. 8 there are 13 pili with 21 phages, and in Fig. 11 there are 21 pili with about 48 phages. Phages and pili were counted on each of 100 poles, and it was found that there were 1,025 virus particles and 332 pili, an average of 3.1 phages for each pilus.

It was difficult to find virions attached to the cells of strain PAO1 in negatively stained preparations of an incubated mixture of PO4 and 3.5-exponential-phase bacteria (MOI 10). The few virus particles which had adsorbed appeared in ones or twos at the poles of the cells. Phages could not be found attached to the pili. With strain PAO68, no virions could be found adsorbed to either cells or pili, which are serologically similar to those of PAO1 (8). These observations will be discussed in relation to the quantitative phage adsorption experiments and EOP determinations. An exhaustive electron microscope examination of adsorption mixtures of strains K/1PO4⁻ and PAO1/PO4⁻ with PO4 failed to show any virions adsorbed to the organisms.

DISCUSSION

Classification of phage PO4. This bacteriophage belongs to the group with long noncontractile tails and double-stranded DNA (4), and in these respects it is unremarkable. Its size,

TABLE 5. *The adsorption of phage PO4 to mutants of P. aeruginosa strains K and PAO1 over 20 minutes*

Strain	PO4·K adsorbed (%)	PO4·PAO1 adsorbed (%)	Pili/pole
K	98.9	94	3.6
K3fla	99.8		4.4
K/PB1	98.4		0.7
K/1PO4⁻	<2		0
K/2PO4⁻	5		0
K3fla /PO4⁻	5		0
K/12Pf 2	<2		0
PAO1	28	63	2.1
PAO68	46	52	3.2
PAO1/PO4⁻	3	4	0
PAO1/PP7⁻	<2	<2	0

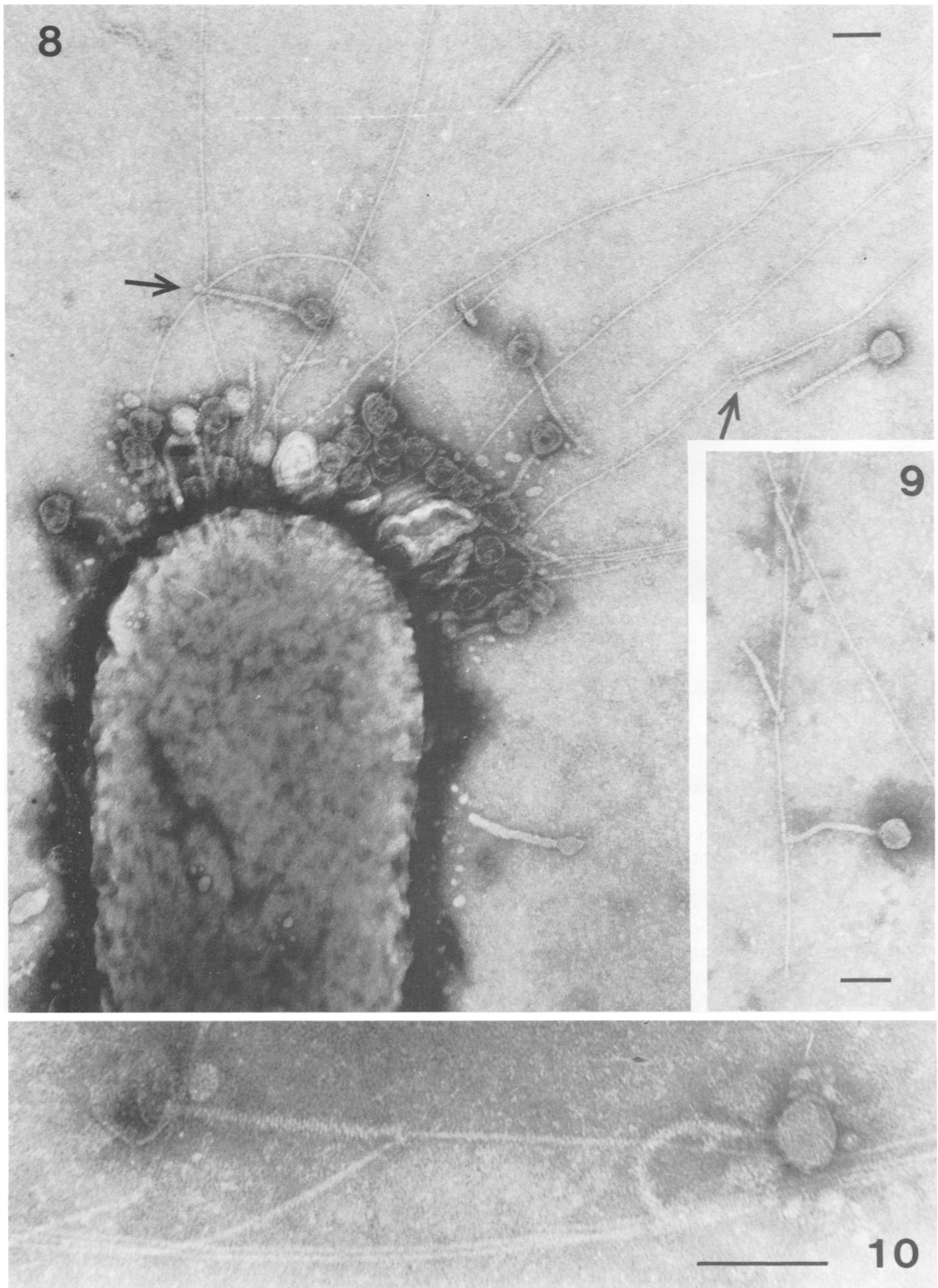

FIG. 8. *PO4 virions adsorbed to the pole of a strain K cell. Arrow mark phages attached to pili. Scale in Figs. 8 through 10: 100 nm. ×75,000.*

FIG. 9. *Virions adsorbed near pilus tip. ×75,000.*

FIG. 10. *Virions adsorbed to pili, one with the tail tip hooked round a pilus. ×195,000.*

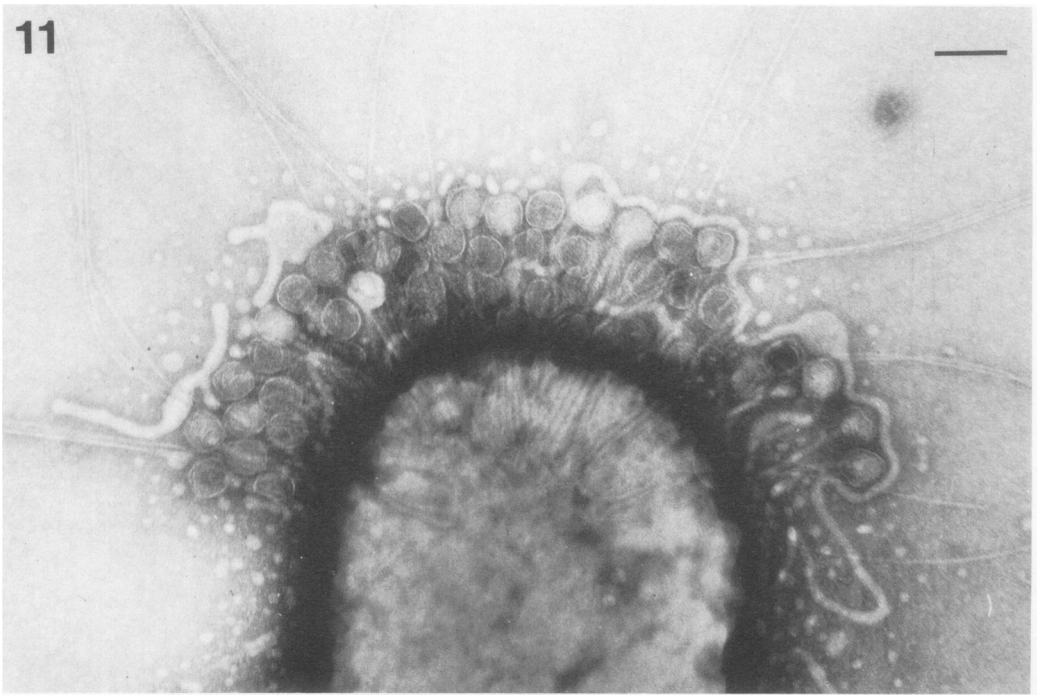

FIG. 11. *Virions adsorbed to the pole of a strain K cell. There are more phages and correspondingly more pili than in the example in Fig. 8. Scale: 100 nm. ×90,000.*

head shape, and other morphological characteristics are similar to many other phages in the group. Even the bar-shaped tail structure, which might be involved in the phage's ability to adsorb to pili, is common among other *Pseudomonas* (3) and *E. coli* phages (4). Spot tests revealed that infection by these phages was independent of the presence of either pili or the F factor.

The question whether PO4 is temperate or virulent requires discussion. On the basis of the curing of phage-producing strains by anti-phage serum (1), it would seem that PO4 is virulent towards strains K and PAO1. Supporting data are provided by its clear plaques, the low frequency (about 13%) of producers, and the inability of mitomycin C to induce a sample of three producer strains. Nevertheless, the possibility cannot be entirely ruled out that the virus exists in its host as a "metavirus," as is the case with filamentous coliphages (13), and not as a true plasmid. In this event, the anti-PO4 serum might also effect a cure by stopping the growth of PO4-producing cells as is reported for the filamentous coliphages (13).

Host range and growth characteristics. Unlike those of *E. coli*, the filamentous and RNA phages of *P. aeruginosa* do not share a common host strain. RNA phage PP7 grows on strain PAO1 (and many derivatives), and Pf grows on strain K. The host range is determined by the nature of the pili, which are serologically different (D. E. Bradley, Can. J. Microbiol., in press). It is of considerable interest that PO4 is able to infect both strains and, as will be discussed, to adsorb to the different pili. The low EOP on strain PAO1, even after the adaptation procedure, may reflect the reduced affinity of the virions for the pili. The situation is to some extent analogous to that of *E. coli* strains bearing the different types of sex pili associated with the plasmids F *lac*, R538-1 *drd*, R1-19, and R100-1 (W. Paranchych, *In* The RNA phage, in press). Each of these plasmids codes for a serologically distinct pilus, like those of PAO1 and K. Nevertheless, the RNA phage R17 can infect cells bearing any of these sex pili, but with varying EOP. Paranchych suggests that a specific protein configuration or component is required in pilin (the pilus protein) to promote phage attachment and that it is present in varying amounts in the different F-like pili. Similar conclusions might be drawn regarding strains K and PAO1, particularly since they are both able to adsorb PO4 with different efficiencies which are related to their respective EOPs.

growth characteristics of infected bacteria. The

curves for strain PAO1 indicate that only a very few cells are lysed. Curves for PAO1 infected with phage PB1 (10) and PP7 (3) showed times between infection and lysis of 30 and 40 min, respectively. PO4 thus has a much longer latent period.

Relationship between piliation and PO4 sensitivity for P. aeruginosa mutants. Table 4 gives results which show that phage PO4 is pilus specific. It is accepted for pilus phages that resistant host mutants are mostly mutants which lack pili (D. E. Bradley, Can. J. Microbiol., in press; 16). Strain PAO68 is exceptional with its nonretractile pili; hence the criterion should be extended to indicate that resistant host mutants can also have defective pili. On strain K and its derivatives, PO4 has the same lytic activity pattern as the filamentous pilus phage Pf, and on strain PAO1 and derivatives, PO4 activity coincides with that of pilus phage PP7. Resistant mutants isolated by using both Pf and PP7 also have no pili and are resistant to PO4, providing further evidence that it is a pilus phage.

Adsorption of PO4. Table 5 shows that PO4 can only adsorb to piliated bacteria, again with the expected exception of strain PAO68. Another point emerges from these figures. The adsorption to strain PAO1 is very inefficient, but is slightly improved by the adaptation procedure. In addition, PO4 grown on strain PAO1 is adsorbed slightly less efficiently by strain K than when the phage is grown on K. This suggests that, by cycling the phage through a different host, one is selecting phage variants which are more compatible with the different pili and rather less compatible with the original pili of strain K. The very low percentage of PO4·K adsorbed by strain PAO1 (28%) suggests that the low EOP of the phage on strain PAO1 (0.001) is due to inefficient adsorption rather than any block in intracellular multiplication. Adsorption to PAO68 does not appear to be greatly affected by selection on PAO1, but this could be masked to some extent by the larger numbers of pili and the fact that they do not retract. The relative inefficiency of PO4 adsorption to strain PAO1 is reflected in electron microscope observations when only a few virions are seen attached to the cell poles.

At first sight, the appearance of adsorbed virions at the poles of sensitive cells might tend to refute the idea of the pilus dependence of PO4, particularly since polar phages have been described (11, 15). However, the phage is frequently seen adsorbed to pili. It was also noted in the electron microscope that virions not actually attached to pili were preferentially located close to them and were usually full. This suggested that many had fallen off during drying. The question is how do the virions reach the cell surface? The alternatives are that they travel down the pili or that the pili retract, pulling them to the surface. The latter is more likely, and an appropriate model for the adsorption and penetration stages of the infective process is proposed. It is suggested that the virions attach tail-first to the pili, which are stimulated to withdraw into the cell. The virus is thus pulled to the surface where secondary receptors may be located. It then injects its DNA. It is unlikely that the pilus acts as a DNA conductor before retraction since full virions are found adsorbed to pili and almost all those at the cell surface are empty. It seems most unlikely that the thin pilus filament would be capable of transporting double-stranded DNA down its length. Thus, the isolation of a double-stranded DNA pilus phage strongly supports the concept of pilus retraction in *P. aeruginosa*.

This model is similar, in most respects, to that proposed for the RNA phage PP7, which is also drawn to the cell surface (6, 8). However, there is a slight difference with PO4 in that there appear to be more phages at the cell surface than pili. With PP7 there was usually one phage on the cell surface at the base of each pilus. It has been reported that the RNA phage PP7 prevents the pilus retracting fully (6, 8), and it is suggested that PO4 does not. In this event, each pilus bearing a single phage would disappear by retracting fully, or alternatively by breaking off, after it had transported the virion to the surface. This would account for the larger numbers of phage particles with respect to pili. Alternatively, each pilus could transport several phages to the surface. In either case the model differs from that proposed for PP7 (6). It is also notable that, like PP7 (7), PO4 adsorption appears to stimulate the growth of pili (9) though not to the same extent.

With the filamentous phage Pf, virions were also found adsorbed to the cell surface at the pole (D. E. Bradley, Can. J. Microbiol., in press), and it was thought that they attached to the pilus tips rather than the sides and were pulled into contact with the cell surface. It was thought that DNA penetration was achieved by some of the virions penetrating the cell in their entirety. A similar process has been proposed for the F-pilus-specific filamentous coliphages (12, 13). It is clear that none of the models for the various pilus phages distinguishes between two possible kinds of pilus retraction: stimulated and spontaneous. In the first case a signal would be transmitted down

the pilus after the adsorption of a bacteriophage, causing the pilus to be disassembled in the cell and hence retracted. With spontaneous retraction, no such signal would be required. The pili would be continually withdrawn and replaced by others, or they could grow out again from the same point once retraction had been completed. Either way, PO4 virions would be efficiently pulled to the cell surface. The hypothesis of stimulated retraction seems rather more likely since PAO1 showed a 50% reduction in pilus length when RNA phages were adsorbed at one per pilus (6, 8). If spontaneous retraction took place, one would expect a greater length reduction of the order of 75%.

The results obtained here are entirely consistent with the proposal that PO4 is a pilus phage. The reasons are (i) that it is seen to adsorb to pili in the electron microscope; (ii) that it attaches to the cell itself at the pole, the only site having pili; (iii) that it cannot infect, nor adsorb to, host mutants which lack pili; (iv) that it causes an increase in piliation on adsorption (9); (v) that it duplicates the behavior of the known pilus phage PP7 in that it adsorbs to but cannot infect a strain of *P. aeruginosa* having a nonretractile pili (strain PAO68). The importance of such a phage lies in its possible use in studying the function of pili. Unfortunately, attempts to isolate an F-pilus-specific phage for *E. coli* have so far proved unsuccessful. Current investigations suggest that this type of bacterial virus may be quite widespread for *P. aeruginosa*.

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