Pilus-dependence of Four *Pseudomonas aeruginosa* Bacteriophages with Non-contractile Tails

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SUMMARY

Four *Pseudomonas aeruginosa* bacteriophages with non-contractile tails were thought to be pilus-dependent because they did not lyse pilus-less host mutants. They were, therefore, subjected to a series of tests based on the properties of known pilus phages. The lytic spectra of the phages and their efficiency of adsorption to various host mutants were compared with those of the RNA pilus phage PP7 and the tailed pilus phage PO4. Electron microscopy was used to locate the sites of adsorption of the candidate phages on sensitive host cells, and on cells of host mutants with non-retractile pili. In all respects, the four isolates behaved like known pilus phages, and it was concluded that they too were pilus-dependent.

A detailed model for the adsorption process of one of the isolates (M6) is proposed on the basis of high-resolution electron micrographs.

INTRODUCTION

A number of pilus-dependent bacteriophages have been found for *Pseudomonas aeruginosa*, including filamentous and RNA-containing types (Feary, Fisher & Fisher, 1964; Bradley, 1966; Takeya & Amako, 1966; Olsen & Shipley, 1973). The subsequent isolation of a pilus phage with a long non-contractile tail (Bradley, 1973a, b) prompted the search for further morphological forms. The present communication describes the testing of 52 isolates to establish unequivocally whether or not they were pilus dependent. Provisional selection was based on the inability of phages to lyse pilus-less host mutants. One important aspect of the subsequent tests involved the use of host mutants carrying non-retractile pili. One mutant was found to be resistant to the RNA pilus phage PP7, and one was resistant to the tailed pilus phage PO4, yet both were able to adsorb their respective viruses. Electron microscopy revealed that both phages attached to the pili well away from the cell surface, whereas with phage-sensitive hosts the virus particles adsorbed at the cell surface. It was concluded that the pili of both mutants were inactive and unable to draw the phage particles to the cell surface to permit nucleic acid penetration, and that they were non-retractile unlike normal host pili (Bradley, 1972b, c; 1974). One of the properties of these mutants is that they have many more pili than their parent strains.

The tests were based on the following criteria: (i) a pilus phage should neither adsorb to nor infect pilus-less host mutants; (ii) it should adsorb to, but not infect, host mutants carrying inactive (non-retractile) but serologically similar pili (Bradley, 1972a, b; 1974);
(3) the majority of host mutants resistant to pilus phages are pilus-less (D. E. Bradley, unpublished observations); (4) the adsorption of virus particles to host pili (or non-retractile pili) should be visible in the electron microscope. With respect to the last criterion, it has been found with the tailed pilus phage PO4 (Bradley, 1973a, b), that the virus particles preferentially adsorb to the poles of sensitive cells, only a few being attached to pili. As has been mentioned above, it is believed that the infecting virus particles are drawn to the cell surface by the retraction of the filaments. However, with the PO4-resistant host mutant carrying non-retractile pili, as in (2) above, many virus particles were attached to the pili but very few to the polar surface (Bradley, 1974). Criterion (4), therefore, refers to pili on both the sensitive host and the resistant mutant. This paper describes the results of the tests obtained with four pilus phage candidates, provisionally selected as mentioned above; their basic morphology is also illustrated.

METHODS

Bacteria. The following strains of Pseudomonas aeruginosa were used: K(ATCC 25102), K/PB1− (strain K resistant to phage PB1, Bradley, 1973b), K/PO4− (pilus-less mutant of strain K resistant to phage PO4, Bradley, 1973b), K/2PfS (mutant of strain K carrying non-retractile pili and resistant to phages Pf and PO4, Bradley, 1974), PAO1 (ATCC 25247), PAO1/PP7− (formerly 1/7, pilus-less mutant of strain PAO1, Bradley, 1972c), PAO68 (carrying non-retractile pili and resistant to phage PP7, Bradley, 1972c). In addition, mutants resistant to each of the candidate viruses were isolated as described below.

Bacteriophages. The following Pseudomonas aeruginosa bacteriophages were used: contractile cell-wall phage PB1 (Bradley & Robertson, 1968), RNA pilus phage PP7 (Bradley, 1966), non-contractile tailed pilus phage PO4 (Bradley, 1973a, b), filamentous phage Pf (Takeya & Amako, 1966), contractile-tailed cell-wall phage 68, one of the P. aeruginosa typing phages used in the Central Public Health Laboratory (CPHL), London, NW9 5HT (Lindberg et al. 1963). We examined 52 other phages for pilus-dependence including 22 in current use at the CPHL for phage typing (see Lindberg et al. 1963; Edmonds et al. 1972; Martin, 1972), 19 isolated at the CPHL from lysogenic strains, and 19 isolated in Greece and used for phage typing (Pavlatou & Hassikou-Kaklamani, 1961).

The four candidates provisionally selected for pilus dependence were all from the collection of the CPHL, with additional details as follows: PE69, isolated from sewage (Deepham Sewage Works, Edmonton, England) by standard methods; C22, isolated by Dr E. H. Asheshov from a lysogenic strain of Pseudomonas aeruginosa (personal communication); C5, originally isolated by Dr M. P. Pavlatou and used by him as a typing phage (Pavlatou & Hassikou-Kaklamani, 1961); M6 is another typing phage used in the CPHL (Lindberg et al. 1963). It has no connexion with the Pseudomonas maltophilia phage described by Moillo (1973), also numbered M6.

Culture media and methods. Oxoid Nutrient Broth was used for all cultures, 2% (w/v) agar being added for plates. Standard double-agar-layer plates contained a final concentration of 0.5% (w/v) agar in the top layer. All bacteria were grown in shake culture at 37°C for the tests.

Preparation of bacteriophage suspensions. High titre phage stocks were prepared either by the confluent lysis of bacterial growth on large (15 cm diam.) double-agar-layer plates followed by extraction with broth for 2 h or as follows. A nutrient agar plate was flooded with a 4 h static culture of the host bacterium, and the excess pipetted off. After drying in air for 10 min, 0.1 ml of phage suspension was spread over the plate. After overnight
Pseudomonas aeruginosa pilus phages

incubation, the phage was extracted with broth. With both methods, bacteria were removed by sedimentation. When required, suspensions were concentrated by high-, then low-speed sedimentation (100000 g for 1 h and 7000 g for 20 min), and finally suspended in broth.

**The spot test for bacteriophage lytic activity.** A loopful of high-titre phage suspension was placed on a double-agar-layer plate of the bacterium under test. Alternatively, 0.02 ml was applied to a plate of bacteria seeded as described above. In both cases, clearing after overnight incubation indicated lytic activity. To avoid anomalous results due to bacteriocin activity, which is particularly prevalent with *Pseudomonas aeruginosa*, phages for spot tests were grown on bacterial strains which did not produce aeruginocins active on the strain under test.

**Isolation of phage-resistant mutants.** Host mutants resistant to three of the candidate phages (M6, PE69, C5) were isolated as follows. Double-agar-layer plates of phage + host mixture were incubated overnight and 14 surface colonies from each were streaked. These were tested for resistance to the isolating phage and to the appropriate known pilus phages for the host strain by the spot test. They were also tested for sensitivity to the wide-range phage PBI. One host mutant for each phage was taken at random for adsorption tests (see below). It was purified by selecting single colonies twice, and checked for resistance to the isolating phage and susceptibility to PBI. Finally it was studied in the electron microscope by antibody labelling (see below) to ensure that it was in the same serological group as the original host. With phage C22, surface colonies were picked from a clear-spot test of the phage on strain PAO1, because the overgrowing bacteria on a confluently lysed plate were not resistant to it. The mutants were numbered K/4PE69, K/5C5, PAO1/3M6 and PAO1/12C22.

**Determination of the efficiency of adsorption of pilus phage candidates to Pseudomonas aeruginosa strains.** Quantitative adsorption tests were carried out as described by Bradley (1974). 3 ml of log phase bacteria at about 2 × 10⁶ cells/ml (estimated using Wellcome opacity tubes, Borroughs Wellcome Ltd., Beckenham) were mixed with 3 ml of phage at about 4 × 10⁷ p.f.u./ml and incubated at 37 °C with shaking for 10 min. After the removal of bacteria by sedimentation, the supernatant fluid was titrated for unadsorbed phage by standard methods. Efficiency of adsorption was calculated as the percentage of phage adsorbed with reference to a blank sample containing no bacteria.

**Electron microscopy.** Specimens of bacterial cells with adsorbed phage particles were prepared as follows. A few drops of a log phase culture (about 4 h) were mixed with sufficient high-titre phage suspension (approx. 10¹³ p.f.u./ml) to give a multiplicity of infection (m.o.i.) of at least 100 (final vol. 0·5 to 1·0 ml). After incubation with shaking at 37 °C for 10 min, the cells were mounted for electron microscopy in one of two ways. For strains PAO1 and PAO68, carbon-coated support grids were floated on the surface of the mixture for 2 to 5 min. They were then washed by floating first on water, then on neutral 0·1 m-ammonium acetate solution. Grids were negatively stained by floating on 0·67 % sodium phoshotungststate solution for a few moments, after which they were dried. Cells of strains K and K/2PFS cannot be mounted satisfactorily by the flotation method, hence sedimentation was used. Carbon-coated grids were placed on the bottom of tapered 10 ml centrifuge tubes and the mixture of phage and bacteria was added (0·2 to 0·5 ml per tube). If necessary, the grid was turned carbon-side upwards, using a wire with a bent tip. After sedimentation at 1000 g for 3 to 5 min in swing-out buckets, the tubes were removed and the grids retrieved by sliding them up the side of the tubes with the bent wire. They were washed free of excess bacteria by immersion in water, then 0·1 m-ammonium acetate, and
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Table I. The lytic activity of the pilus phage candidates and known pilus phages on strains of Pseudomonas aeruginosa

<table>
<thead>
<tr>
<th>Lytic activity of bacteriophages*</th>
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<tbody>
<tr>
<td>Cell-wall phage</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Strain</td>
</tr>
<tr>
<td>K</td>
</tr>
<tr>
<td>K/PBI</td>
</tr>
<tr>
<td>K/2PFS</td>
</tr>
<tr>
<td>K/1PO4</td>
</tr>
<tr>
<td>K/4PE69</td>
</tr>
<tr>
<td>K/5C5</td>
</tr>
<tr>
<td>PAO1</td>
</tr>
<tr>
<td>PAO68</td>
</tr>
<tr>
<td>PAO1/PP7</td>
</tr>
<tr>
<td>PAO1/3M6</td>
</tr>
<tr>
<td>PAO1/12C22</td>
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</tbody>
</table>

* As indicated by the spot test; + indicates clearing and sensitivity to a phage, – no clearing and resistance.
† + indicates presence of pili, (+) non-retractile pili, – no pili.

0.67 % sodium phosphotungstate for negative staining. Electron micrographs were calibrated using 264 nm polystyrene latex spheres.

Antibody-labelling for electron microscopy (Lawn, 1967) was used to check that phage-resistant host mutants were in the correct serological group and were not contaminants. Grids with mounted bacteria were floated on, or immersed in, a 25 % aqueous solution of serum for 30 s; the serum was prepared against parental-type flagella and pili (Bradley, 1972b). After washing and negative staining as above, the coating of the flagella with antibodies was checked in the electron microscope. Phage-resistant mutants isolated using the candidate phages were checked for pili by observing 200 poles on negatively stained cells. Tests for the serological relatedness of similar phages were carried out by standard methods.

RESULTS

Screening test for candidate phages

The 52 phages mentioned above (see Methods) were examined by the spot test on the following strains of Pseudomonas aeruginosa: K, K/1PO4–, PAO1, PAO1/PP7–. Four of the phages tested (C22, M6, PE69 and C5) failed to lyse pilus-less strains (K/1PO4–, PAO1/PP7–) but lysed one or both of the piliated wild-type strains (K, PAO1). These phages were tested more extensively as described below.

Lytic activity of bacteriophages as shown by the spot test

The results are shown in Table I. The piliation of each strain of Pseudomonas aeruginosa is included for correlation with the host range of each phage. The wide host-range cell-wall phage PBI, with the resistant bacterial mutant K/PBI–, are included for comparison with the known pilus phages (PP7, PO4, Pf) and the candidates. The following relevant points emerge. (a) The candidates, like known pilus phages, do not necessarily lyse both strains K and PAO1. (b) No known pilus phage can lyse any pilus-less strain or a strain with non-retractile pili. The candidates follow this pattern without exception. (c) The
Table 2. Adsorption of pilus-phage candidates to Pseudomonas aeruginosa strains over 10 minutes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Piliation*</th>
<th>C22</th>
<th>M6</th>
<th>PE69</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K/2PhS</td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K/4PE69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K/5C5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAO1</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAO1/3M6</td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PAO1/12C22</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* + indicates presence of pili, (+) non-retractile pili, - no pili.

phage-resistant mutants isolated using the candidates (for further details see below) correspond in their phage sensitivity patterns to those isolated against known pilus phages, and are resistant to all the candidates.

In summary, the candidates and their host mutants behave in exactly the same way as the known pilus phages and their corresponding resistant mutants.

Adsorption of bacteriophages to Pseudomonas aeruginosa

The adsorption test results in Table 2 show that in all cases piliated strains of bacteria, regardless of whether or not the pili are retractile, are able to adsorb the pilus phage candidates. Pilus-less host mutants do not adsorb them significantly. These results are in accordance with criteria (1) and (2) above (see Introduction). It is to be noted that with all the candidates, the efficiency of adsorption by resistant strains with non-retractile pili is rather less than by sensitive strains with retractile pili. Similar results were obtained with the known pilus phages Pf and PO4 (Bradley, 1974).

Resistant mutants isolated using the candidate phages

One of the criteria for pilus dependence in a bacteriophage is that it should be easy to isolate host mutants resistant to known pilus phages using the candidate virus. As has been described, a number of colonies derived from plates confluent lyed by the candidate phages were tested by the spot test. The numbers of mutants resistant to the isolating phages only are compared to the numbers resistant to both isolating phages and the known pilus phages for the original hosts, in Table 3. A typical phage which is not pilus-dependent is included for comparison (PB1). None of the mutants isolated using PB1 is resistant to known pilus phages, in contrast to the mutants isolated using the candidate phages. Resistance to two unrelated pilus phages (Pf and PO4 for strain K derivatives, and PP7 and PO4 for strain PAO1 derivatives) is a strong indication of the loss of, or a defect in, the pilus receptors (including inability to retract). Of the four mutants selected for further study (Tables 1, 2), none was found to have any pili when 200 poles of each were checked in the electron microscope.
Table 3. Proportion of phage-resistant mutants which were resistant to known pilus phages* as well as the candidate phage used for isolation

<table>
<thead>
<tr>
<th>Isolating phage</th>
<th>Host strain</th>
<th>Number of mutants tested</th>
<th>Number resistant to isolating phage†</th>
<th>Number resistant to isolating and pilus phages*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBI‡</td>
<td>K</td>
<td>14</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>C22</td>
<td>PAO1</td>
<td>13</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>M6</td>
<td>PAO1</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>PE69</td>
<td>K</td>
<td>14</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>C5</td>
<td>K</td>
<td>14</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

* The known pilus phages for the parent strain: Pf and PO4 for strain K, PP7 and PO4 for strain PAO1.
† Excluding contaminants indicated by resistance to phage PBI, or for PBI-resistant mutants, phage 68.
‡ The contractile phage PBI (not pilus-dependent) is included for comparison.

Electron microscopy

Phage PBI

This contractile bacteriophage adsorbs to the cell wall of a sensitive bacterium. Fig. 6 illustrates this and is included for comparison with Fig. 1, 7, 10 and 15. Unlike the pilus phage candidates, the adsorbed PBI virus particles are evenly distributed over the cell surface. The pili of the bacterial strain illustrated (PAO68) are non-retractile, and no virus particles could be found attached to them.

Phage C22

As might be expected from its low efficiency of adsorption, few C22 particles could be seen attached to the sensitive host, strain PAO1 (Fig. 1, arrow). The virus particles adsorbed preferentially to the cell pole. It was not possible to locate phages attached to the pill of strain PAO68, to which C22 adsorbed with even less efficiency (see Table 3). However, it was found that the phage preparation from a confluently lysed plate of PAO1 contained many pili with virus particles or headless tails attached (Fig. 2, 3). In appearance, the phage is identical to PO4 (Fig. 4, 5), having a 60 nm head with a hexagonal outline and a long (190 nm) tail with the typical bar-shaped structure at the tip. With C22, this structure is more fragile than that of PO4, readily breaking up into a mass of fibres (Fig. 2). Fig. 4 and 5 show more or less intact tail tips. Phage C22 is not inactivated by anti-PO4 serum.

Phage M6

Fig. 7 shows how the virus particles adsorb specifically to the pole of the sensitive strain PAO1. It is to be noted that they are all empty, presumably having injected their DNA, and there are no visible pili. In a few instances, however, virus particles could be found adsorbed to PAO1 pili: in Fig. 8, details of the tail tip and the way in which it is attached to the pilus can be seen. The bar-shaped structure is similar to that found on the pilus phage PO4 (Bradley, 1973a, b) but is more stable and resolves into sets of three fibres, each with a knob at the tip. Two or three similar fibres extend from the pointed tail tip. The micrograph also shows the manner in which the virus particle is attached to the pilus: the fibres appear to be wrapped round it. This feature will be discussed in detail below. It is also evident that the head has burst, losing its contents, but the tail still contains nucleic acid since it is solid, unlike the headless tail in the same micrograph. When adsorbed
Pseudomonas aeruginosa *pilus phages*

Fig. 1. Phage C22 virus particle (arrowed) attached to the pole of the sensitive strain PAO1 (m.o.i. 500).
Fig. 2. Headless tail of C22 particle attached to the tip of a pilus, a common feature in an extract from a confluent lysed plate of strain PAO1.
Fig. 3. Complete C22 virus particle adsorbed to two pili in the same preparations as Fig. 2.
Fig. 4. Empty C22 virus particle showing hollow tail and bar-shaped structure at the tip.
Fig. 5. Intact C22 virus particle showing solid tail and hexagonal head.
Fig. 6. The contractile cell-wall phage PB1 adsorbed to strain PAO68. The virus particles are evenly distributed round the periphery of the cell (m.o.i. 400).

Fig. 7. Phage M6 adsorbed to the sensitive strain PAO1. Empty virus particles are preferentially located at the cell pole (m.o.i. 1000).

Fig. 8. An M6 virus particle adsorbed to a PAO1 pilus. The head has been squashed and lost its DNA, but the way in which the phage tail is attached to the pilus can be seen.
Fig. 9. Phage M6 adsorbed to the resistant strain PAO68, which has non-retractile pili. Virus particles are distributed along the lengths of the pili and have retained their DNA in most cases (m.o.i. 1000).
Fig. 10. Phage PE69 adsorbed to the sensitive strain K. Empty virus particles are attached preferentially to the cell pole (m.o.i. 100).

Fig. 11. A PE69 virus particle attached to a pilus of the resistant strain K/2PfS, which has non-retractable pili.

Fig. 12. Phage PE69 adsorbed to strain K/2PfS. The full virus particles are attached to the pili where they are at their greatest density (m.o.i. 100).

Fig. 13, 14. PE69 virus particles showing general morphology.
Pseudomonas aeruginosa pilus phages

Fig. 15. Phage C₅ adsorbed to the sensitive strain K, empty virus particles being attached to the pole (m.o.i. 150).

Fig. 16. Phage C₅ virus particle attached to a pilus of the resistant strain K/²PFS (non-retractile pili).

to strain PAO68 with non-retractile pili, M₆ virus particles are evenly distributed along the lengths of the filaments (Fig. 9). Most of the phage particles are full, and only a few are located near the cell pole. Structurally, M₆ is typical of its morphological group, with an oblong head (85 nm x 55 nm) and a striated tail (150 nm).

Phage PE69

When adsorbed to the sensitive host strain K, phage PE69 attached to the pole (Fig. 10). The virus particles are empty. Only a few could be found attached to pili (Fig. 11). In Fig. 12, PE69 virus particles are shown adsorbed to strain K/²PFS non-retractile pili. While the phages are full, they tended to prefer the pilus bases, but only a few were adjacent to the cell surface. Fig. 13 and 14 show the general structure of the phage. It has a short non-contractile tail (45 nm) with a collar and a rather indistinct tail tip (Fig. 13). Both these structures appear to break up easily (Fig. 14). The head (87 nm) is probably octahedral and is large for a phage of this morphological group.

Phage C₅

As with M₆ and C₂₂, phage C₅ adsorbs to its sensitive host, PAO₁, at the pole (Fig. 15). It could not be found adsorbed to the pili of this strain, but with strain K/²PFS it was
Fig. 17. Phage C5 virus particles adsorbed to a cell of the resistant strain K/2P/S (non-retractile pili). They are adsorbed at random along the pili and retain their DNA (m.o.i. 200).

attached to the non-retractile pili (Fig. 16, 17) and not to the poles. Like PE69, this phage belongs to the group with short non-contractile tails, but is smaller (65 nm head) and has a very short tail (approx. 15 nm).

DISCUSSION

Suggested models for the adsorption and penetration of the known pilus phages are briefly reviewed here to provide a basis for comparison of the present results.

The RNA phage PP7 is thought to adsorb firmly to the sides of the pili of its host. The pili then retract, drawing the virus particles to the cell surface where they prevent further
Fig. 18. Diagrammatic representation of the adsorption and penetration of phage M6. (a) A full virus particle and the pilus, which passes through a hole in the cell envelope (thick layer: cell wall; thin layer: cell membrane) to a site which both polymerization and depolymerization of pilus protein are thought to occur. (b) The virus particle adsorbs to the side of the pilus by means of its lateral tail fibres (two sets only are shown for clarity), probably wrapping two sets of them round the pilus filament (arrowed). (c) The pilus retracts, pulling the phage tail tip to its base; DNA injection would be initiated on contact. (d) The pilus continues to retract, either slipping through (shown here) or breaking off the lateral tail filaments, until it is fully withdrawn. DNA penetration is completed.

retraction (Bradley, 1972b); RNA injection then takes place. In the electron microscope, cells with adsorbed phage have many pili, each with a virus particle at its base. RNA phage particles adsorbed to strains with non-retractile pili are randomly distributed along the filaments and are not located at the bases.

With the tailed pilus phage PO4, virus particles again adsorb to the sides of the pilus and are drawn to the cell surface as with PP7. However, the bond between the phage and the pilus is thought to be sufficiently weak to allow the pilus to continue retracting. In the electron microscope, the phage particles thus appear to be adsorbed to the poles with relatively few pili visible (Bradley, 1973b; 1974). Since DNA penetration takes place on
contact with the cell wall, the virus particles are empty. With non-retractile pili, the virus particles are again distributed along the pili and are full, since DNA injection has not been triggered; few are close to the cell pole.

Turning to the present results, all of the C22-resistant mutants are also resistant to the known pilus phages (Table 3). C22 has the same patterns of adsorption and lytic activity on sensitive and resistant strains as the established pilus phages (Tables 1, 2).

As with C22, all the M6-resistant mutants are also resistant to known pilus phages (Table 3). In the electron microscope, M6 is clearly attached to the poles and to the pili of its host in a similar manner to the known pilus phage PO4. It also adsorbs to the non-retractile pili of the resistant strain PAO68 in very large numbers (Fig. 9).

With phage PE69, only 9 out of 14 resistant mutants were also resistant to known pilus phages. However, it should be pointed out that phage resistance need not necessarily be achieved by the alteration or loss of receptors, but can also occur when there is a block at some stage of intracellular multiplication. Despite having the correct adsorption characteristics to strain K/2PFS, it does not appear to be convincingly attached along the whole length of the non-retractile pili (Fig. 12), appearing mostly near the cell where the filaments are more concentrated. However, the virus particles remain full unlike those adsorbed to the pole of the sensitive host strain K (Fig. 10).

Phage C5 behaved exactly like the known pilus phage PO4 in all the tests. Since each test is in itself a strong indication of pilus-dependence, it is considered that all the phages are true pilus phages.

The electron micrographs of M6 are more detailed than those obtained previously with PO4 (Bradley, 1973a, b), which has a similar tail structure. Because of its greater stability, it is possible to see the way in which the M6 tail is attached to the pilus (Fig. 8), and a detailed model for the penetration process can now be suggested. As shown in Fig. 18, the virus particle has several sets of three laterally extending filaments with knobs at their tips near the end of the tail; the number of sets cannot be accurately estimated in the electron microscope, but general appearance (contrast, etc.) suggests three or four. The same suggestions apply to the filaments at the point of the tail. In the model illustrated, the virus particle attached to the side of the pilus by wrapping two sets of lateral filaments around it (Fig. 18b, arrowed). This would account for the considerable thickening of the pilus at the point of attachment (Fig. 8). The tail tip appears to be held away from the pilus by the filaments at the point. Retraction of the pilus then occurs; the virus particle is drawn to the cell wall (Fig. 18c), where the pilus is thought to pass through a small hole (Bradley, 1972b). This hole may incorporate the somatic receptor which triggers nucleic acid penetration. It is thought that the pilus continues retracting until completely withdrawn. All that remains is an empty virus particle at the cell pole without any visible pilus beside it (Fig. 18d).

Finally, we may consider the implications of the results in relation to the structure of the phages. It is interesting that pilus-dependence is not restricted to long-tail types, since PE69 and C5 resemble coliphage T3 in their morphology. So far, no contractile bacteriophages have been found to be pilus-dependent. Phage M6 resembles a previous isolate PB2 (Bradley, 1966) and the Pseudomonas aeruginosa transducing phage F116 (Slayter, Holloway & Hall, 1964), which itself appears to be a pilus phage (Pemberton, 1973). However, we tested F116 and found that it was not serologically related to M6.

Tailed pilus phages are clearly useful in studying the property of retraction in pili. Also it follows from the results described here, that if an established pilus phage appears to adsorb to the pole of a sensitive strain, then the pili on that strain are retractile. The
Pseudomonas aeruginosa pilus phages

behaviour of the pilus phage candidates is valuable support for the concept of pilus retraction in Pseudomonas aeruginosa. It is suggested that mutants with non-retractile pili could be used to study the mechanism of pilus-mediated bacterial conjugation.

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REFERENCES

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