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An immunological study of the pili of *Pseudomonas aeruginosa*

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SUMMARY

An attempt was made to correlate serological relationships determined by the pili, the flagella and the O-somatic antigens of *Pseudomonas aeruginosa*, and to make a preliminary assessment of use of the pilus antigen as an epidemiological marker. A method is described for the preparation of antiserum specific for the 'normal' PSA pili of *P. aeruginosa*. A high titre of pilus antibodies was obtained by immunizing rabbits with mutants whose pili had lost their ability to retract into the cell. The 'normal' form of the organism, with retractile pili, was poorly agglutinated by high-titre anti-pilus serum, but suspensions of it that had been treated with osmium tetroxide showed greatly increased agglutinability. Antibody labelling for electron microscopy was used to determine the serological relations of pili and of flagella for *P. aeruginosa* strains belonging to different serological groups as defined by O-somatic antigens. The distribution of pilar and flagellar antigens among strains was not correlated with the O-somatic serotype. A strain of *P. aeruginosa* carrying a drug-resistance plasmid had fewer 'normal' PSA pili than the background strain.

Pseudomonas aeruginosa normally possesses thin polar pili (fimbriae) which constitute one of the classes of heat-labile antigens of the organism. A previous study of the flagellar antigens (Pitt & Bradley, 1975), which are also heat-labile, was an essential preliminary to the development of a useful typing system based on heat-labile antigens. The present work extends this to the pilus antigens. The principle object is to define serological differences determined by pili, flagella, and heat-stable O-somatic antigens. In order to make this comparison, it has been necessary to develop a method for preparing specific antisera against pili, free from flagellar and O-somatic antibodies.

Many studies have been made of *P. aeruginosa* pili; two basic types of pili have so far been established: (1) the 'normal' polar pili mentioned above (Bradley, 1966), to be called PSA pili for convenience and (2) the non-polar pili determined

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by the intergeneric P-group drug-resistance plasmid RP1, called RP1 pili (Bradley, 1974*a*; C. H. To & C. C. Brinton, in preparation). Non-polar pili have also been found in association with the *P. aeruginosa*-specific drug-resistance plasmid R130 (L. E. Bryan, University of Alberta, Canada, personal communication), but they are still under study and little is known about them. Since the present work is principally aimed at evaluating the usefulness of pilus antigens as serological markers, emphasis has been placed on PSA pili, though obviously the presence of R-factor-determined pili cannot be ignored.

There are two ways of observing the serological relationships of bacterial appendages such as pili: either qualitatively by the direct observation of adsorbed antibodies in the electron microscope (Lawn, 1967), or quantitatively by conventional agglutination tests. In this work we have used the first method to define different serological types of pilus, and the second to study in detail the reactions of antibodies specific to the pilus.

Although the injection into rabbits of formolized suspensions of *P. aeruginosa* results in the formation of flagellar antibody in high titre, the antibody response to the pili is poor (Pitt & Bradley, 1975). The pilus is in the form of a long thin filament consisting of polymerized pilus protein or pilin. It has been demonstrated (Bradley, 1972*a*) that under certain influences, such as chemical action or bacteriophage adsorption (see below), the filaments withdraw into the cell, the pilin probably being depolymerized by a mechanism at the base. Certain *P. aeruginosa* mutants that lack the ability to retract their pili (Bradley, 1972*a*, 1974*b*) have many more filaments than do normal strains. We now show that these mutants are valuable in producing high-titre antiserum. Other mutants without pili have been isolated (Bradley, 1972*b*), and absorption of sera with these organisms removes antibodies to other components of the cell.

Certain bacteriophages use *P. aeruginosa* pili as receptors (Bradley, 1966, 1973*a*, *b*, 1974*a*; Bradley & Pitt, 1974). One aspect of the present work has been to determine whether the phage-sensitivity pattern varies with pili of different serological types.

MATERIALS AND METHODS

Bacteria and bacteriophages

Details of the strains of *P. aeruginosa* used are given in Table 1. Bacteriophages were as follows:

(1) RP1-specific phages: PRRI – an RNA phage (Olsen & Thomas, 1973) supplied by Dr R. H. Olsen; Pf3 – a filamentous phage (Stanisich, 1974) supplied by Dr V. A. Stanisich.

(2) Phages specific for PSA pili: PP7 – an RNA phage (Bradley, 1966); Pf – a filamentous phage (Takeya & Amako, 1966); PO4 (Bradley, 1973*b*); M6, C22, PE69, C5 (Bradley & Pitt, 1974), and F116 (Pemberton, 1973), with non-contractile tails.

Phage PS1 was obtained from Dr P. H. Clarke (University College, London), and phage P18 was isolated as a temperate phage from *P. aeruginosa* strain 18S; both are shown to be pilus-dependent phages in this paper.

Table 1. *List of strains of* Pseudomonas aeruginosa

Strain	New designation*	Description	Origin
PAO1	PAO *pil*^{r} *fla*$^{+}$	Wild type, retractile PSA pili	ATCC 25247
PAO68	PAO *pil*^{nr} *fla*$^{+}$	PAO1 mutant, non-retractile PSA pili	B. W. Holloway (Bradley, 1972*a*)
PAO1/PO4^{-}	PAO *pil*$^{-}$ *fla*$^{+}$	Pilus-less mutant of PAO1	Bradley (1973*b*)
PAO2605	PAO (RP1^{+})	PAO1 derivative with R factor RP1	B. W. Holloway
K	K *pil*^{r} *fla*$^{+}$	Wild type, retractile PSA pili	ATCC 25102
K/2PfS	K *pil*^{nr} *fla*$^{+}$	K mutant, non-retractile PSA pili	Bradley (1974*b*)
K3*fla*$^{-}$	K *pil*^{r} *fla*$^{-}$	K with retractile PSA pili, no flagella	Bradley (1973*a*)
K/1PO4^{-}	K *pil*$^{-}$ *fla*$^{+}$	Pilus-less mutant of K	Bradley (1973*a*)
K3*fla*$^{-}$/PO4^{-}	K *pil*$^{-}$ *fla*$^{-}$	Pilus-less mutant of K, no flagella	Bradley (1973*b*)
18S	—	Background strain for R factor RP1	M. H. Richmond
18S RP1	18S (RP1^{+})	18S carrying RP1	M. H. Richmond
H1	—	Wild type, PSA pili	Central Public Health Lab.
M6	—	Wild type, PSA pili	Central Public Health Lab.
PAC1	—	Wild type, PSA pili	P. H. Clarke (Brown & Clarke, 1972)

* Designation devised for this paper: *pil*^{r} = retractile PSA pili, *pil*^{nr} = non-retractile PSA pili, *pil*$^{-}$ = no pili, *fla*$^{+}$ = flagella, *fla*$^{-}$ = no flagella.

Culture media and solutions

Oxoid nutrient broth CM1 was used throughout for both liquid and plate cultures, the latter containing either 1 or 2% agar. Bacteriophages were propagated (Adams, 1959) and extracted from confluently lysed double-agar-layer plates (soft agar layer, 0·5% agar in broth).

Saline was NaCl 0·85% (w/v) in distilled water. Osmium saline was 0·1% OsO_4 (w/v) in saline. Formol saline was formaldehyde 40% diluted to 0·2% with saline.

Spot tests for bacteriophage activity

A drop of high-titre phage suspension was placed on the surface of a double-agar-layer plate of the bacterial strain under test. After overnight incubation, clearing indicated sensitivity and no clearing resistance. When the phage was propagated on a host that formed an aeruginocin (bacteriocin for *P. aeruginosa*) active on the strain under test, the following procedure was adopted. An initial test was carried out as described above, then soft agar from the cleared area was removed with a loop and resuspended in about 0·5 ml. of broth. This was used in a

second test. In this way, insufficient aeruginocin was carried over to the second test to produce anomolous clearing, but adequate phage was present to form plaques on a sensitive strain.

Agglutination tests

Bacterial vaccines were prepared as follows. Four-hour broth cultures were used to seed five 9 cm agar plates which were incubated at 37° C. for 18 hr. The bacteria were harvested in 10 ml. of saline, washed once by centrifugation, and resuspended to a concentration of *ca.* 2×10^9 bacteria/ml. as estimated by opacity (opacity tube no. 2, Wellcome Opacity Tubes, Burroughs Wellcome Ltd., Beckenham, Kent).

To prepare antisera, rabbits were first given bacterial suspensions killed at 100° C. for 2 hr. Two 1 ml. intravenous injections were given at an interval of 3 days. Five days later, injections of live cells of the same strain were given at 3- or 4-day intervals. The first four (0·25 ml., 0·5 ml., 1·0 ml. and 1·5 ml. respectively) were given subcutaneously, and the last two doses, each of 2·0 ml., intravenously. The rabbits were test-bled at 3- or 4-day intervals during immunization and exsanguinated 3 days after the end of the immunization course. Serum was separated by centrifugation and stored at −20° C. after the addition of merthiolate (0·2 %) as a preservative.

Sera were absorbed as follows. Cells of the absorbing strain harvested in formol saline from 9 cm agar plates were deposited by centrifugation at 10,000 ***g*** for 30 min. Suspensions to be used for the removal of anti-O antibodies were boiled for 2½ hr. before centrifugation; those used to absorb anti-flagellar antibodies were not. To absorb an antiserum, 1 ml. of packed cells was thoroughly mixed with an equal volume of serum, incubated at 37° C. for 2 hr. and left for a further 3 hr. at 4° C. The bacteria were removed by centrifugation, and the serum was passed through a membrane filter.

Suspensions for agglutination were prepared by suspending the overnight growth from an agar plate or slope in *ca.* 5 ml. of saline or osmium saline. The opacity was adjusted by dilution to correspond to opacity tube no. 2, the estimated number of bacteria being *ca.* 2×10^9 cells/ml. Agglutination tests were performed by mixing 0·3 ml. volumes of the bacterial suspension with equal volumes of doubling dilutions of serum in Dreyer's tubes. A control tube of saline plus suspension was included. The tests were incubated at 50° C. for 2 hr. and read with a ×2 magnifying lens while obliquely illuminated.

Electron microscopy

The specific antibody-labelling of PSA pili and flagella for electron microscopy was performed by the method of Lawn (1967). Bacteria were mounted by centrifuging cells directly onto carbon-coated electron-microscope support grids at about 1500 ***g*** (Bradley, 1973*a*). The grids were immediately transferred to suitably diluted antiserum containing antibodies to both pili and flagella prepared as for agglutination tests. After 30 sec. they were washed twice in water and negatively stained with sodium phosphotungstate solution 1 % (w/v).

For the identification and study of RP1 pili, a slightly different procedure was used. RP1 pilus antigen was prepared as follows. Each of eight 15 cm double-agar-layer plates of *P. aeruginosa* strain 18S RP1 was extracted with 10 ml. of broth for 3 hr. After the removal of bacteria by low-speed centrifugation, the resulting pilus suspension was saturated with ammonium sulphate. The floc was skimmed off and dialysed against broth.

After removing debris by low-speed centrifugation, the suspension was injected intravenously into rabbits (eight injections of *ca.* 2·0 ml. over 1 month). Two final injections at days 44 and 45 were followed by bleeding at day 54. Bacteria were labelled with antibodies for electron microscopy by incubating 1·8 ml. of a log-phase broth culture (*ca.* 5×10^8 cells/ml.) with 0·2 ml. of antiserum for about 2 hr. at 37° C. Cells were mounted by centrifugation and negatively stained.

Pilus counts were carried out in the electron microscope on about 100 poles of cells of the bacterial strain under test.

RESULTS

Serological tests

Antisera prepared against mutants of strains K and PAO with non-retractile pili, K *pil*^{nr} *fla*$^+$ and PAO *pil*^{nr} *fla*$^+$ respectively, were absorbed with boiled homologous cells to remove O antibodies and then tested by the tube-agglutination method with all members of the K and PAO series (see Table 1). The K *pil*^{nr} *fla*$^+$ antiserum was then absorbed in turn with formolized cells of the following: K *pil*^{r} *fla*$^+$ (wild type K), K *pil*$^-$ *fla*$^+$ and K *pil*$^-$ *fla*$^-$. The PAO *pil*^{nr} *fla*$^+$ antiserum was absorbed with similarly treated cells of PAO *pil*^{r} *fla*$^+$ (wild type PAO), and PAO *pil*$^-$ *fla*$^+$.

The results show (Table 2) that high agglutination titres of homologous strains were obtained with sera prepared against *pil*^{nr} strains. These titres were in part due to flagellar antibodies, because the *pil*$^-$ *fla*$^+$ strains of K and PAO were also agglutinated to high titre (2560 and 5120 respectively) by the respective sera.

Absorption with the corresponding *pil*$^-$ *fla*$^+$ variants removed the flagellar antibody and revealed titres of anti-pilus antibody of 5120 for strain PAO when tested with *pil*^{nr} suspensions. The titres of anti-pilus antibody in sera made against the respective wild strains with retractile pili were 160 or less (Pitt & Bradley, 1975). Absorption with *pil*^{r} or *fla*$^+$ forms resulted in little reduction of titre of anti-pilus antibody, and *pil*^{r} suspensions were agglutinated only at low titre by absorbed sera that had high titres of anti-pilus antibody as evidenced by agglutination of *pil*^{nr} mutants. It appears that the *pil*^{r} form has reduced ability to absorb pilus antibody and is poorly agglutinable by it.

As would be expected, bacteria without pili but with flagella (*pil*$^-$ *fla*$^+$ were unable to remove any pilus antibodies, and absorption of serum K *pil*^{nr} *fla*$^+$ with K *pil*$^-$ *fla*$^-$ did not affect the high titres of the piliate or flagellate strains.

The absorbed sera were also titrated with suspensions of strains grown at 44° C., so preventing the formation of flagella (T. L. Pitt, unpublished). The pilus-antibody titres were unaffected, indicating that pilus-antigen synthesis was not inhibited at

Table 2. *Agglutination titres with absorbed and unabsorbed sera, and with mutants differing in pilar and flagellar constitution*

	Bacterial suspensions							
	K	K	K	K	K	PAO	PAO	PAO
Antisera	*pil*^{nr} *fla*$^{+}$	*pil*^{r} *fla*$^{+}$	*pil*^{r} *fla*$^{-}$	*pil*$^{-}$ *fla*$^{+}$	*pil*$^{-}$ *fla*$^{-}$	*pil*^{nr} *fla*$^{+}$	*pil*^{r} *fla*$^{+}$	*pil*$^{-}$ *fla*$^{+}$
K *pil*^{nr} *fla*$^{+}$	5120	2560	80	2560	80	< 20	< 20	< 20
ab K *pil*^{r} *fla*$^{+}$	1280	160	80	< 20	< 20	—	—	—
ab K *pil*$^{-}$ *fla*$^{+}$	5120	80	40	< 20	< 20	—	—	—
ab K *pil*$^{-}$ *fla*$^{-}$	5120	2560	80	2560	< 20	—	—	—
PAO *pil*^{nr} *fla*$^{+}$	< 20	< 20	< 20	< 20	< 20	10,240	5120	5120
ab PAO *pil*^{r} *fla*$^{+}$	—	—	—	—	—	640	< 20	< 20
ab PAO *pil*$^{-}$ *fla*$^{-}$	—	—	—	—	—	5120	320	20

—, Not tested. ab, Absorbed.

Table 3. *Agglutination titres with bacterial suspensions fixed and not fixed with osmium tetroxide**

	Bacterial suspensions							
	K	K	K	K	PAO	PAO	PAO	PAO
Antisera	*pil*^{nr} *fla*$^{+}$	*pil*^{nr} *fla*$^{+}$ OsO_4	*pil*^{r} *fla*$^{-}$	*pil*^{r} *fla*$^{-}$ OsO_4	*pil*^{nr} *fla*$^{+}$	*pil*^{nr} *fla*$^{+}$ OsO_4	*pil*^{r} *fla*$^{+}$	*pil*^{r} *fla*$^{+}$ OsO_4
K *pil*^{nr} *fla*$^{+}$	5120	10,240	80	5120	—	—	—	—
ab K *pil*$^{-}$ *fla*$^{+}$	5120	10,240	40	5120	—	—	—	—
PAO *pil*^{nr} *fla*$^{+}$	—	—	—	—	10,240	10,240	5120	10,240
ab PAO *pil*$^{-}$ *fla*$^{+}$	—	—	—	—	10,240	10,240	320	10,240

—, Not tested. ab, Absorbed.

* Fixation was with 0·1 % OsO_4 for 30 min. at room temperature.

Table 4. *The antibody-labelling* of pili and flagella detected by electron microscopy*

Strain	Serological group†	Labelling of the indicated strain by antiserum against no.: PAO1 P‡	PAO1 F‡	18S P	18S F	K P	K F	H1 P	H1 F	M6 P	M6 F	PAC1 P	PAC1 F
PAO1	2	+	+	−	+	−	−	−	−	+	−	−	−
PAO2605§	2	+	+	−	+	−	−	−	−	+	−	−	−
18S	2	−	+	+	+	−	−	−	−	−	−	−	−
K	6	−	−	−	−	+	+	+	−	−	−	−	−
H1	1	−	−	−	−	+	−	+	+	−	−	−	+
M6	11	+	−	−	−	−	−	−	−	+	+	−	−
PAC1	3	−	−	−	−	−	−	−	+	−	−	+	+

* +, Appendage coated with antibodies; −, no antibodies present. No distinction is made between light and heavy labelling since strengths of antisera vary.

† Serological group number with reference to heat stable O-somatic antigens.

‡ P, Pilus; F, flagellum.

§ Not examined for RP1 pili in this table, only the labelling of PSA pili being recorded.

44° C. The titre of the flagellar antibodies dropped to 20 and 40 when estimated with suspensions of the pilus-less flagellate strains K *pil*$^-$ *fla*$^+$ and PAO *pil*$^-$*fla*$^+$ respectively.

The effect of fixation with osmium tetroxide

Low concentrations of osmium tetroxide were thought to inhibit pilus retraction (Bradley, unpublished). This hypothesis was tested as follows. The piliate cultures of the K and PAO series were suspended in freshly prepared 0·1 % OsO_4 in saline for 30 min. at room temperature. The cells were washed three times by centrifugation, and resuspended in saline. The titre of agglutination of strain K *pil*^{r} *fla*$^-$ by the homologous anti-pilus antiserum was 40 before and 5120 after treatment with osmium tetroxide (Table 3). A similar effect was observed with strain PAO *pil*^{r} *fla*$^+$.

Electron microscopy of antibody-labelled pili and flagella

Bacterial cells treated as described with antiserum containing antibodies to pili and flagella were studied in the electron microscope. The visible coating of the pili and flagella with antibodies was noted for all possible combinations of antiserum and bacterial strain, and the results are given in Table 4. An additional column shows the O-serological group (Habs, 1957) of each strain. Cross-labelling of pili or flagella was not confined to one O group; the flagella of strains H1 (O-group 1) and PAC1 (O-group 3) were serologically related. Similarly, the pili of PAO derivatives (O-group 2) are related to those of M6 (O-group 11), and those of K (O-group 6) to H1 (O-group 1). Similarly members of the same O-group did not necessarily carry serologically related pili, as shown by the antibody-labelling

Table 5. *Sensitivity of piliate and non-piliate mutants of* Pseudomonas aeruginosa *to phages Ps*1 *and P*18 *shown by the spot test*

Strain	Lysis* by phage		
	Ps1	P18	PE69
K *pil*^{r} *fla*$^{+}$	+	+	+
K *pil*^{r} *fla*$^{-}$	+	+	+
K *pil*^{nr} *fla*$^{+}$	−	−	−
K *pil*$^{-}$ *fla*$^{+}$	−	−	−

* +, Clearing; −, no clearing of the bacterial lawn by phage action.

method; there was no cross-labelling of the pili of the O-group-2 strains 18S, PAO1 and PAO2605, even though the flagella were labelled (Plate 1).

The labelling of an RP1 pilus with specific antibody is shown in Plate 2. The micrograph illustrates a PSA pilus without antibodies, together with a much shorter antibody-labelled pilus on the same cell. The same antiserum had previously been used to label RP1 pili on *Escherichia coli* (Bradley, 1974*a*); the pilus shown is thus identified as being determined by the R factor.

Sensitivity of P. aeruginosa *strains to pilus-dependent bacteriophages*

Bacteriophage spot tests were used to see whether pilus-phage sensitivity patterns differed between strains having serologically related PSA pili and whether the acquisition of RP1 pili changed the phage-sensitivity pattern. Two new pilus phages, Ps1 and P18 (Bradley, in preparation), were used in addition to those previously described (Bradley, 1966, 1973*a*, *b*; Pemberton, 1973; Bradley & Pitt, 1974). Table 5 shows that, like the known pilus phage PE69, phages Ps1 and P18 can form plaques only on strains carrying retractile pili. In the electron microscope, however, both phages were seen to be adsorbed to the PSA pili (retractile or non-retractile) of strains K *pil*^{r} *fla*$^{+}$ and K *pil*^{nr} *fla*$^{+}$ (not illustrated).

The results of the spot tests on strains of different serological groups are shown in Table 6. It is notable that, in the case of strain 18S, the acquisition of RP1 changes the phage-sensitivity pattern. The loss of PSA pilus phage sensitivity is probably due to the change in piliation shown in Table 7. With the remaining strains, a heterogeneous set of sensitivity patterns is shown. This emphasizes that strains with serologically related pili, such as K and H1, do not share the same pilus-phage sensitivity pattern.

*The effect of factor RP*1 *on the number of PSA pili per pole*

In order to determine whether or not the presence of the drug resistance plasmid RP1 has any effect on the number of PSA pili present on a cell, pilus counts were made on strains 18S and 18S RP1 and also on strains PAO1 and PAO2605 (RP1^{+}). The results in Table 7, which are discussed below, show that there is a definite reduction in piliation in strain 18S, but not in the PAO derivative, when RP1 is present.

Table 6. *Sensitivity of* Pseudomonas aeruginosa *strains to bacteriophages shown by the spot test*

Strain	Serological O-group	Lytic activity of bacteriophages*: RP1-specific PRR1	RP1-specific Pf3	PSA pilus-specific PP7	PSA pilus-specific Pf	PSA pilus-specific PO4	PSA pilus-specific PE69	PSA pilus-specific C5	PSA pilus-specific M6	PSA pilus-specific F116	PSA pilus-specific P18	PSA pilus-specific C22	PSA pilus-specific Ps1
PAO1 (wild type)	2	−	−	+	−	+	−	+	+	+	−	+	−
PAO2605 (RP1+)	2	+	+	+	−	+	−	+	+	+	−	+	−
18S (wild type)	2	−	−	−	−	−	+	−	−	−	−	+	+
18S (RP1+)	2	+	+	−	−	−	−	−	−	−	−	−	−
K	6	−	−	−	+	+	+	+	−	+	+	+	+
H1	1	−	−	−	−	+	+	+	+	+	−	+	+
M6	11	−	−	+	−	−	+	+	+	+	+	+	+
PAC1	3	−	−	+	−	+	+	+	+	+	+	+	+

* +, Clearing; −, no clearing.

Table 7. *Piliation* of strains of* Pseudomonas aeruginosa

Strain	Pili/pole	Poles piliated (%)
18S	2·2	77
18S RP1	0·4	25
PAO1	0·4	23
PAO2605 (RP1$^+$)	0·7	32

* No distinction was made between PSA and RP1 pili, which were infrequent, because the antibody-labelling required to distinguish between them makes counting difficult due to the pili tangling.

DISCUSSION

Serological reactions

It is evident that pili do stimulate an antibody response, and that some 'minor' antibodies previously attributed to flagella may have been anti-pilar. A high titre of serum antibody against the pilus results from the immunization of rabbits with mutants that have non-retractile pili. To obtain a specific anti-pilus serum, it would be necessary to use a vaccine strain that was also non-flagellate, or to remove the flagella or antibody by absorption with a mutant. Our preliminary experiments suggest that growth at 44° C. inhibits the production of flagella; whether this inhibition is sufficiently complete to prevent antibody production is not yet known.

Treatment with osmium tetroxide greatly increases the agglutinability by anti-pilus serum of 'wild' strains that have retractile pili. The non-retractile pili of strains K *pil*^{nr} *fla*$^+$ and PAO *pil*^{nr} *fla*$^+$ are serologically indistinguishable from the osmium-tetroxide-fixed retractile pili of their parent strains K *pil*^{r} *fla*$^+$ and PAO *pil*^{r} *fla*$^+$. Fixation thus appears to prevent pilus retraction. We do not yet know whether high-titre antisera can be obtained with fixed cells as an alternative to organisms with non-retractile pili.

The effect of the R factor RP1 on piliation

The effect of a change in the number and type of pili present on an organism on its serological characteristic has not yet been completely explored. The acquisition of factor RP1 by strain 18S results in a fivefold reduction in the number of PSA pili present. In addition, a different surface antigen has been added in the form of the RP1 pilus, which can coexist with PSA pili on the same cell. As a result, the bacteriophage sensitivity pattern is basically changed by the acquired sensitivity to RP1-specific phages, and the resistance or decreased sensitivity to PSA pilus phages. The second effect does not occur with the two PAO strains, the wild type and PAO2605 (RP1$^+$), studied here. B. W. Holloway (personal communication) reports that some clones of PAO derivatives acquiring factor RP1 can lose sensitivity to the PSA pilus phages PP7 and F116, as is the case with strain 18S (RP1$^+$) and the pilus phages PE69, C22 and Ps1. He indicates that this effect is not general but varies from clone to clone. In all these examples it seems likely that the PSA pili are lost or considerably reduced in number. Drug-resistance

factors, which are becoming increasingly widespread, may therefore have a significant influence on the way in which any typing system based on pilus antigens is applied.

Pilus retraction

The degree to which a pilus reacts with its homologous antiserum depends upon whether or not it is retractile. Previously, only derivatives of strains PAO and K have been shown to have retractile pili (Bradley, 1972*a*, 1974*b*), but we have demonstrated that strains 18S, H1, M6 and PAC1 are all sensitive to one or more of a series of bacteriophages (PP7, PO4, PE69, C5, M6, C22) all of which require retractile pili for infection (Bradley, 1972*a*, 1973*b*; Bradley & Pitt, 1974). By inference, the PSA pili of these four strains are also retractile, and it seems likely that this characteristic is widespread. The results of our agglutination tests provide additional supporting evidence for the retractile nature of PAO and K pili.

Potentialities of the pilus antigen as an epidemiological marker

We have shown that there is considerable serological diversity among both pili and flagella of *P. aeruginosa* (see Table 4 and Pitt & Bradley, 1975). In the six strains examined, four different flagellar antigens and the same number of pilus antigens were found. This serological heterogeneity among the pilus antigens suggests that they might be useful as epidemiological markers. It is also evident that the pilus antigen is not related to the O-somatic or flagellar antigens as judged both by antibody labelling for electron microscopy, and by agglutination tests.

It can be seen from Tables 4 and 6 that the pattern of sensitivity to lysis by pilus phages is different for all strains except those derived from the same parent (PAO derivatives), even though two strains may have serologically related pili. Thus, pilus-phage sensitivity is a more sensitive indication of differences in PSA pili. However, the apparent ease with which pili may be lost, either through the acquisition of a plasmid, or by mutation, suggests that the use of pilus phages in any phage-typing system is of limited value. However, the pilus antigen might be useful as a subsidiary to typing by means of O and H antigens, which appear to be more stable. One factor influencing the application of the pilus antigen as an epidemiological marker is that PSA pili might be coded for by a transferable plasmid, as is the case with RP1 pili. However, preliminary attempts to transfer PSA pili by conventional mating techniques, and also by mobilization with factor RP1, have failed (D. E. Bradley, unpublished). PSA pili may be determined by a chromosomal gene or some form of non-transferable plasmid, but there is at present no strong evidence favouring either of these alternatives.

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EXPLANATION OF PLATES

Plate 1

P. aeruginosa strain PAO2605 was labelled with antiserum to strain 18S, both being in serological group 2 as determined by O-somatic antigens. The antibodies to strain 18S PSA pili have not labelled the PSA pili of strain PAO2605, but the flagellum is heavily coated with 18S flagellar antibodies. × 140,000.

Plate 2

P. aeruginosa strain PAO2605 labelled with antiserum to strain 18S RP1 which contains antibodies to RP1 pili but not to pili (PSA type) of PAO derivatives. Antibodies have coated the pilus determined by the drug resistance plasmid (arrow), but not the PSA pili, showing that the two types can coexist on the same cell. × 140,000.

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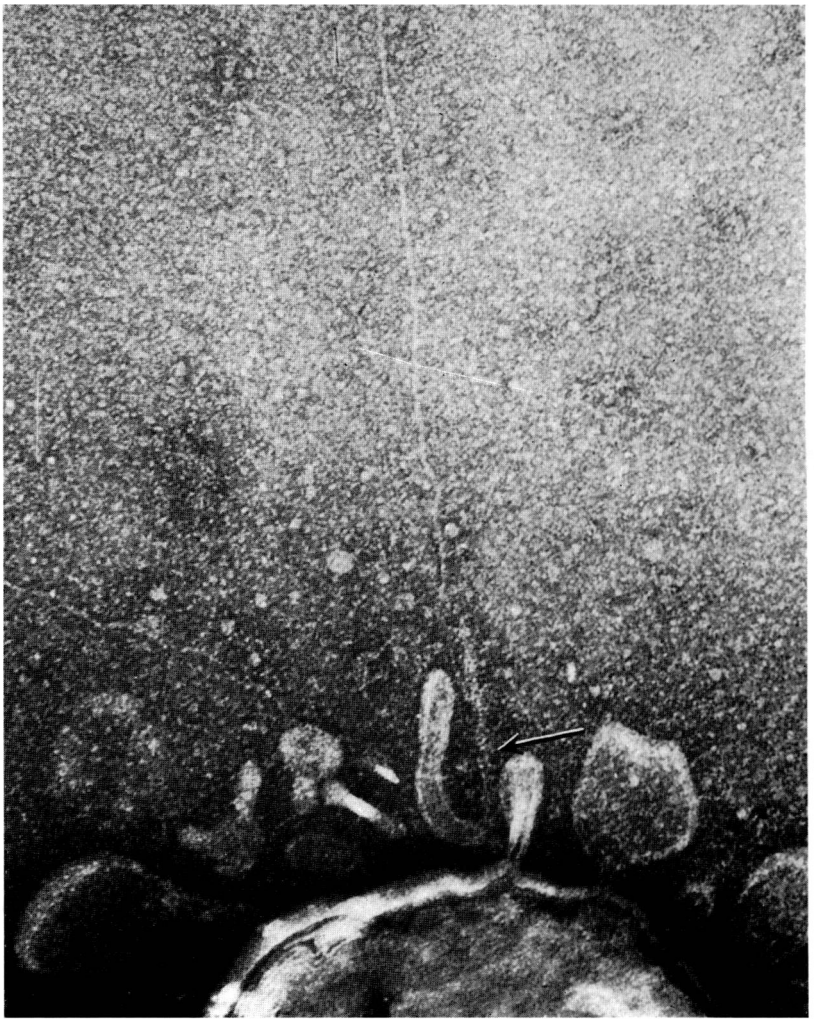

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