Surface Appendages Similar to Fimbriae (Pili) on *Pseudomonas* Species

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SUMMARY

Twenty-two strains of *Pseudomonas*, representing 15 species, were examined by electron microscopy, utilizing the techniques of negative contrast staining and to a lesser extent shadowing and ultrathin sectioning. Fimbria-like appendages were found on cells of 12 strains, representing 8 species. Polar, fimbria-like filaments were observed on *Pseudomonas aeruginosa*, *P. acidovorans*, *P. testosteroni*, *P. maltophilia*, *P. alcaligenes* and *P. solanacearum*. Peritrichous filaments were found on *P. multivorans* and *P. fragi*.

INTRODUCTION

Bacterial fimbriae or pili are filamentous appendages of bacterial cells which differ from flagella in a number of ways. Fimbriae do not exhibit the sinuous form characteristic of bacterial flagella and they are apparently not related to the motility of flagellated bacteria (Brinton, 1965; Duguid, Anderson & Campbell, 1966). Fimbriae are often thinner than flagella and may differ from flagella in detailed fine structure (Duguid, Smith, Dempster & Edmunds, 1955; Brinton, 1967). Like flagella, fimbriae may be of polar or peritrichous distribution, depending on the genus or species of bacterium examined (Houwink & van Iterson, 1950). There is no convincing evidence at present of the occurrence of fimbriae in other than Gram-negative bacteria.

Much recent work has been concentrated on fimbriae of the enterobacteria and, with notable exceptions (e.g. Schmidt, 1966; Tweedy, Park & Hodgkiss, 1968), the polar flagellates have been relatively neglected. Fimbriae have been reported on only four *Pseudomonas* species; *Pseudomonas aeruginosa* (Houwink & van Iterson, 1950; Bradley, 1965, 1966); *P. echinoides* (Marx & Heumann, 1962; Heumann & Marx, 1964), *P. piscicida* (Hansen, Ingebritsen & Weeks, 1963) and *P. multivorans* (Tweedy *et al.* 1968). It would seem that the majority of *Pseudomonas* species have not been examined systematically using appropriate methods. In order to extend existing knowledge of the occurrence of fimbriae in *Pseudomonas*, fifteen physiologically diverse species of the genus were examined by electron microscopy as part of a survey of the filamentous appendages found on the surface of pseudomonad cells. The techniques of negative contrast staining and to a lesser extent shadowing and ultrathin sectioning were used.

Like Tweedy *et al.* (1968), we do not wish to imply through use of the term fimbriae for filamentous appendages other than flagella on pseudomonads that such appendages are comparable in every respect to the fimbriae of other organisms. We also use

'fimbriae' (Duguid *et al.* 1955) rather than 'pili' because it has priority and because we accept the other arguments (Duguid, 1966; Duguid & Anderson, 1967) in favour of this term.

METHODS

Organisms and media. The organisms examined in this study, as listed in Table 1, were grown in either peptone yeast extract broth (Difco bacto peptone, 10.0 g.; Difco bacto yeast extract, 5.0 g.; NaCl, 5.0 g.; distilled water 1 l.; pH 7.2), sucrose peptone broth (sucrose, 20.0 g.; Difco bacto peptone, 5.0 g.; MgSO₄·7H₂O, 0.25 g.; K₂HPO₄, 0.5 g.; distilled water 1 l.; pH 7.0 to 7.2), 0.1 % lactate mineral salts medium (lactic acid, 1 g./l.; standard mineral base of Stanier, Palleroni & Doudoroff, 1966), or 0.1 % lactate mineral salts medium supplemented with 0.025 % DL-methionine (for *Pseudomonas maltophilia* only).

Table 1. *Pseudomonas* species examined for fimbriae

Strain no.	Organism	Culture collection no. or source
85*	<i>P. aeruginosa</i>	ATCC 14216; NCIB 9685
80*	<i>P. aeruginosa</i>	ATCC 10145; CCEB 481; NCIB 8295; NCTC 10332
67*	<i>P. fluorescens</i>	CCEB 488-A; ATCC 15456; NCIB 9886
62*	<i>P. fluorescens</i>	ATCC 13525; NCIB 9046; NCTC 10038
64*	<i>P. chlororaphis</i>	NCIB 9392; ATCC 9446; NRRL B-560
70*	<i>P. aureofaciens</i>	NCIB 9030; ATCC 13985; NRRL B-1576; Delft E. III. 9.25.1
86*	<i>P. putida</i>	CCEB 520; NCIB 9887
83*	<i>P. putrefaciens</i>	NCIB 8615
72*	<i>P. acidovorans</i>	Den Dooren De Jong strain 7; NCIB 9681; ATCC 15668
81*	<i>P. testosteroni</i>	NCIB 8955; ATCC 11996; NRRL B-2611
75*	<i>P. oleovorans</i>	ATCC 8062; NCIB 6576
58*	<i>P. alcaligenes</i>	ATCC 14909; NCTC 10367
0346	<i>P. multivorans</i>	Isolated from forest-litter, Mt. Nebo, Queensland, Australia, in May 1968
036B	<i>P. multivorans</i>	Isolated from tomato stems from Cairns, Queensland, Australia in October 1965
56*	<i>P. fragi</i>	ATCC 4973; CCEB 387; NCIB 8542; NRRL B-25
0147	<i>P. maltophilia</i>	From Commonwealth Serum Laboratories, Parkville, Victoria
61*	<i>P. maltophilia</i>	ATCC 13637; NCIB 9203; NCTC 10257
017A	<i>P. solanacearum</i>	From bacterial wilt of potato, isolated, April 1965. Biotype II
002A	<i>P. solanacearum</i>	From bacterial wilt of tomato, April 1965. Biotype III
003A	<i>P. solanacearum</i>	From bacterial wilt of ginger, April 1965. Biotype IV
450	<i>P. stizolobii</i>	NCPPB 450; W. H. Burkholder PS 23 isolated from <i>Trifolium repens</i> in U.S.A., 1954
0268A	<i>P. stizolobii</i>	Isolated from blackened stems of <i>Vicia sativa</i> cultivar Golden Tares, April 1967. From Wollongbar Agric. Res. Station, N.S.W. Dept Agric.

* Culture obtained from the *Pseudomonas* Working Party.

Organisms to be examined by negative contrast staining or shadowing in the survey for fimbriae were grown through various serial subcultures in 6 × ½ in. test tubes containing 5 or 10 ml. medium and incubated aerobically at 28°. Cells for ultrathin sectioning were grown in 100 ml. medical flats containing 10 ml. of 0.1 % lactate mineral salts medium and incubated aerobically at 28° on their wide sides. The exact ages of each culture examined are listed in the 'Explanation of Plates'. Initial inocula in all cases were from stock agar slope cultures. Purity checks were performed on all

cultures examined by plating out from the culture itself or from the washed cell suspension prepared from the culture (see below).

Negative contrast staining. Cells to be examined were harvested by centrifugation, washed once in sterile distilled water before final resuspension in the wash fluid. A 1 % solution of sodium phosphotungstate (pH 6.0, 7.0 or 7.4) containing bovine plasma albumin to 0.03 % was employed as a negative contrast stain. Negative contrast preparations were usually made by applying a drop of washed bacterial suspension to a carbon-coated copper grid. After allowing up to 3 min. for adherence of cells to the carbon film to take place, excess fluid was removed with filter paper. A drop of negative contrast stain was then applied to the grid and excess fluid again removed. The grid was then transferred to the electron microscope specimen chamber where final drying was allowed to take place. In some cases the negative contrast agent was applied to the grid first (see below).

Shadowing. A drop of washed bacterial suspension (prepared in the same manner as for negative contrast staining, with the exception that in some cases 0.1 % formalin was used as a wash fluid) was applied to a carbon-coated grid and excess fluid removed. The preparation was then shadowed with platinum/carbon. The angle of shadowing was not determined.

Ultrathin sectioning. After 26 hr growth in 100 ml. medical flats containing 10 ml. of 0.1 % lactate mineral salts medium incubated aerobically at 28°, cells were harvested by centrifugation. Pelleted cells were fixed with 4 % (w/v) glutaraldehyde buffered to pH 6.2 with 0.1 M-sodium cacodylate (Sabatini, Bensch & Barrnett, 1963). Fixation was allowed to proceed for 18 hr at room temperature. Cells were then washed in 0.2 M-sucrose solution buffered to pH 6.2 in 0.1 M-cacodylate buffer and postfixation was carried out in osmium tetroxide overnight. After postfixation, cells were washed in 0.5 % uranyl acetate for 1 hr. Dehydration was performed via a series of ethanol solutions and the pellet was embedded in Epon 812 by a method similar to that of Luft (1961). Sections were cut from the resulting block on a Porter-Blum ultramicrotome. Sections were poststained with 5 % (w/v) uranyl acetate for 15 min. and lead citrate (Reynolds, 1963) for 2 min.

Electron microscopy. A Siemens Elmiskop IA was used at accelerating voltages of 80 kV or 100 kV.

Standard procedures for obtaining statistically analysable fimbrial diameter measurements. Organisms were grown for 48 hr in $6 \times \frac{1}{2}$ in. tubes containing 5 ml. of peptone yeast extract broth and incubated aerobically at 28°. Cells were harvested by a standard method and purity checks performed. All preparations were negatively stained with 1 % sodium phosphotungstate (pH 7.0) containing 0.03 % bovine plasma albumin, applied by a standardized procedure. All micrographs of standard preparations were taken at an instrumental magnification of $\times 80,000$. Measurement of fimbrial diameters was made from prints enlarged $\times 2$, using a Peak Scale Lupe 7 x (Kellner type) micrometer with a linear graticule. For determination of means and standard deviations, thirty diameters were measured in each case.

RESULTS

Survey for fimbria-like appendages in Pseudomonas species. Negatively stained preparations of all the strains listed in Table 1 were examined exhaustively in the

electron microscope for fimbriated cells. Among the criteria used to distinguish fimbriae were: (i) position (i.e. surface appendages); (ii) shape (i.e. showing no regular undulations); (iii) diameter (i.e. thinner than flagella); (iv) substructure (i.e. a substructure differing from that of flagella of the same species); (v) distribution (only if fimbrial distribution differed from the flagellar distribution of the same species). Results of the survey are summarized in Table 2. At least 12 out of the 22 strains examined, representing 8 of 15 species, definitely possessed fimbria-like appendages. Appendages thinner than flagella were seen on one strain of *Pseudomonas stizolobii* (strain 0268 A) but these filaments were of variable diameter and seemed to taper in some cases. They were only seen in very old cultures and there is a definite possibility that they are an artifact resulting from autolytic effects or represent slime material.

Table 2. *Occurrence of fimbriae in Pseudomonas species examined by negative contrast staining*

Bacterial strain	Fimbriae observed	Distribution
<i>P. aeruginosa</i> (85)	+	Polar
<i>P. aeruginosa</i> (80)	+	Polar
<i>P. fluorescens</i> (67)	—	.
<i>P. fluorescens</i> (62)	—	.
<i>P. chlororaphis</i> (64)	—	.
<i>P. aureofaciens</i> (70)	—	.
<i>P. putida</i> (86)	—	.
<i>P. putrefaciens</i> (83)	—	.
<i>P. acidovorans</i> (72)	+	Polar
<i>P. testosteroni</i> (81)	+	Polar
<i>P. oleovorans</i> (75)	—	.
<i>P. alcaligenes</i> (58)	+	Polar
<i>P. multivorans</i> (0346)	+	Peritrichous
<i>P. multivorans</i> (036B)	+	Peritrichous
<i>P. fragi</i> (56)	+	Peritrichous
<i>P. maltophilia</i> (0147)	+	Polar
<i>P. maltophilia</i> (61)	—	.
<i>P. solanacearum</i>		
biotype II (017 A)	+	Polar
biotype III (002 A)	+	Polar
biotype IV (003 A)	+	Polar
<i>P. stizolobii</i> (0268 A)	?	?
<i>P. stizolobii</i> (450)	—	.

In a number of strains, attempts to demonstrate fimbriae gave negative results, even after examination of cells grown by several different subculturing procedures and/or in different media. Negative results such as those obtained must be interpreted cautiously for a number of reasons, however (see Discussion), and do not necessarily mean that the organisms are genotypically incapable of synthesizing fimbriae. Among those strains in which fimbriae were found, fimbriation of all cells in a sample was never observed. Usually the majority of cells examined in a preparation were not fimbriated. This perhaps emphasizes the dangers of misinterpreting negative results and the necessity for exhaustive scanning of preparations in a survey for fimbriation.

Polar and peritrichous distributions of fimbriae on individual cells were observed. Where distribution was polar, fimbriae were found on the flagellated pole only, except in the case of strain 85 of *Pseudomonas aeruginosa* and possibly strain 002 A of

P. solanacearum. Bipolar fimbriae were sometimes found on cells of strain 85 of *P. aeruginosa*. Distribution of fimbriae on pseudomonad cells cannot be related in any predictable way to flagellar distribution, apparently, since all the species of *Pseudomonas* examined possessed polar flagella while cells of at least two species possessed peritrichous fimbriae.

In no case did fimbriae on *Pseudomonas* species display a sinuous outline with regular undulations. Fimbriae (especially those of polar distribution) were sometimes seen to be slightly curved or irregularly bent in certain portions. It may be that these filaments are flexible rather than rigidly straight; distortion may result from stress during drying in the course of preparation.

Table 3. *Diameters of fimbriae found on Pseudomonas species by negative contrast staining*

<i>P. aeruginosa</i> (85)	54 ± 14 Å
<i>P. aeruginosa</i> (80)	52 ± 14 Å
<i>P. multivorans</i> (0346)	62 ± 5 Å
<i>P. solanacearum</i> (017 A)	49 ± 9 Å
<i>P. solanacearum</i> (002 A)	55 ± 10 Å
<i>P. solanacearum</i> (003 A)	60 ± 9 Å
<i>P. testosteroni</i> (81)	40 ± 8 Å
<i>P. alcaligenes</i> (58)	65 ± 20 Å

Values for measured diameters of fimbriae (30 diameters measured for each value) are given as the mean ± the standard deviation. Measurements for these values were of fimbriae on cells grown and negatively stained by a standard procedure (see Methods).

Although there may be error in determination of the maximum number and length of fimbriae on individual cells, due to possible breakage of fimbriae, some general observations can perhaps be made. The maximum number of fimbriae observed on either strain of *Pseudomonas multivorans* was greater than 100/cell. About 30 fimbriae/cell was the maximum observed on *P. fragi*. Among those strains with polar fimbriae, however, more than 10 fimbriae/cell were never observed (10/cell were only observed on strain 85 of *P. aeruginosa*). The maximum fimbrial length observed never exceeded 1.7 µ on any strain.

The diameters of fimbriae seen in negative contrast preparations are given for several strains in Table 3. They are all below the diameters observed for the flagella of these strains, and are also below the range of published diameters of unsheathed flagella, i.e. 120 Å (Hoeniger, 1965) to 220 Å (Richter & Kress, 1967).

Substructure of fimbriae seen in negative contrast preparations. Fimbrial substructure was clearly discerned in only a few species. In most cases substructure was not visible or very obscure.

The fimbriae of *Pseudomonas testosteroni* and *P. fragi* exhibited substructures of a periodic nature suggesting that they are composed of repeating subunits of some kind (Pl. 1, fig. 2, Pl. 5, fig. 10).

Dark central lines were observed in the fimbriae of *Pseudomonas multivorans* (Pl. 3, fig. 6) and *P. fragi* (Pl. 5, fig. 10), when seen in negative contrast preparations. The central regions were 13 to 19 Å wide in both species. It is perhaps significant that dark central lines were never seen in the polar fimbriae of other species. Bradley (1966) stated that the fimbriae of *P. aeruginosa* which he observed in negative contrast

preparations appeared to be hollow in places. This was not observed in the fimbriae of either strain of *P. aeruginosa* examined in this study,

The flagella of the *Pseudomonas* species examined were either sheathed or unsheathed. Sheathed flagella were found only on strains of *P. stizolobii* (Fuerst & Hayward, 1969). Where substructure of unsheathed flagella was visible, it usually consisted of an aggregate of globular subunits (e.g. Pl. 5, fig. 10) or a number of dark longitudinal lines alternating with light ones (e.g. Pl. 1, fig. 1), perhaps corresponding to the A and B forms of Lowy & Hanson (1965).

Table 4. *Published reports of fimbriae on bacteria with polar flagella*

Bacterial strain	*Diameter	Distribution	References
<i>Vibrio cholerae</i> (sic)	72 Å	Uniform	Tweedy, Park & Hodgkiss (1968)
<i>V. eltor</i>	79 Å	Uniform	Barua & Chatterjee (1964); Tweedy <i>et al.</i> (1968)
<i>Caulobacter</i> spp.	40 Å	Polar	Schmidt (1966)
<i>Aeromonas hydrophila</i>	.	Peritrichous	Page (1962)
<i>A. liquefaciens</i>	79 Å	Peritrichous	Tweedy <i>et al.</i> (1968)
<i>Photobacterium splendidum</i>	.	Peritrichous	Houwink & van Itersen (1950)
<i>Pseudomonas pyocyanea</i> (<i>P. aeruginosa</i>)	.	Polar	Houwink & van Itersen (1950)
<i>P. aeruginosa</i>	45 Å	Polar	Bradley (1965, 1966)
<i>P. echinoides</i>	50 Å	Polar	Marx & Heumann (1962)
	(shadowed)		Heumann & Marx (1964)
<i>P. multivorans</i>	86 Å	Peritrichous	Tweedy <i>et al.</i> (1968)
	(shadowed)		
<i>P. piscicida</i>		Peritrichous	Hansen, Ingebritsen & Weeks (1963)

* All diameter measurements are from negative contrast preparations unless otherwise stated.

Results of shadowing and ultrathin sectioning. Shadowed preparations were made using both strains of *Pseudomonas multivorans*. Results with respect to distribution of fimbriae confirmed those from negative contrast preparations. However, dimensions and appearances of the filaments were different from those in negative contrast preparations because of the evaporated metal deposit and perhaps because of drying *in vacuo*. Dimensions of fimbriae are above those which would be expected by extrapolation from negative staining results according to the predictions of Thornley & Horne (1962) and Tweedy *et al.* (1968). However, in this study the shadowing material used was different from those used by those workers and the shadowing angle may likewise have been different.

Plate 4, fig. 8 shows cells of strain 0346 of *Pseudomonas multivorans* in what appears to be transverse section. Extending from the periphery of one of the cells are about 15 straight or slightly curved filamentous structures, 31 to 78 Å wide. Another cell in the same micrograph appears to possess similar appendages. These filaments should not be confused with a finger-like extension on one of the cells which does not appear to be a rigid structure and is 94 to 235 Å wide. It may represent a 'bleb' of cell wall or membrane material seen in thin section. The thin filaments occur all around the periphery of one of the cells seen in section, and may be appendages which are peritrichously distributed on the unsectioned cell. Similar filaments were seen on other sectioned cells of the same strain. Penetration of the filaments into the cell sections was obscured in most cases. The filaments did not display clearly definable substructure in these sections. It would seem likely that these filaments represent the same type of

appendage as the peritrichous fimbriae seen in negative contrast and shadowed preparations of this strain.

DISCUSSION

Earlier reports of fimbriae on polarly flagellated bacteria are given in Table 4; there are records of fimbriae on only four species of *Pseudomonas*, two of which have been re-examined here. In this study, fimbriae were found on eight species of *Pseudomonas*, six of which had not been examined before by negative contrast staining. Tweedy *et al.* (1968) state that it has been the experience of one of them in electron microscopic work that fimbriae are possessed by only a few species of bacteria with polar flagella. However, the number of species examined to reach this conclusion was not stated. The results presented here indicate that the ability to produce fimbriae may be a more common feature of pseudomonads than had previously been realized.

Any consideration of the occurrence of fimbriation among different bacterial species must include the interpretation of negative results. The absence of fimbriae on cells of a bacterial strain is a result which may be interpreted in several ways. Not all strains of a species may be genotypically capable of producing fimbriae. Even if they were genotypically fimbriate, organisms may not have been grown under conditions enhancing fimbriation or selecting for fimbriated cells. Another important factor may be examination at a stage of growth when the proportion of fimbriated cells is highest. However, consistently negative results among closely related strains may have some significance. For instance, fimbriae were not observed on *Pseudomonas fluorescens* (strains 62, 67), *P. chlororaphis*, or *P. aureofaciens*. Stanier *et al.* (1966) consider that *P. chlororaphis* and *P. aureofaciens* should be incorporated into the species *P. fluorescens* as biotypes. However, confirmation of these results using other strains is required.

No attempt was made in this study to correlate the presence of fimbriae with haemagglutinating activity. Haemagglutinating activity is not necessarily associated with fimbriae and there are some types of fimbriae without haemagglutinating properties of any kind (Duguid *et al.* (1966). It is also possible that some types of fimbriae may have adhesive properties but because of restricted distribution on the cell (e.g. monopolar) are unable to confer haemagglutinating properties (Heumann & Marx, 1964). Because of these difficulties most attention was given to electron microscopy in this study. However, preliminary work on haemagglutinating properties of pseudomonads has confirmed the results of Tweedy *et al.* (1968) with respect to the mannose-sensitive haemagglutinating properties of *P. multivorans*.

The results of Houwink & van Iterson (1950) on the distribution of fimbriae of *Pseudomonas pyocyanea* (*P. aeruginosa*), which were obtained from shadowed preparations, were confirmed in this study by negative contrast staining. Bradley (1966) could not observe the fimbriae of *P. aeruginosa* by negative contrast methods unless cells examined had been treated with a specific RNA bacteriophage. In this study fimbriae were found readily by negative contrast staining of *P. aeruginosa* which had not been deliberately infected with phage. Bradley (1966) found that the diameter of fimbriae of *P. aeruginosa* observed in phage-infected preparations was about 45 Å, which is similar to the fimbrial diameter measurements for both strains of *P. aeruginosa* examined in this study (i.e. 54 ± 14 Å and 52 ± 14 Å).

Tweedy *et al.* (1968) found numerous, short, straight fimbriae on cells of *Pseudomonas multivorans* NCIB 9691 by shadowing. We have observed numerous peritrichous

fimbriae on two other strains of *P. multivorans* (i.e. 036B and 0346) by both negative contrast and shadowing methods. In the case of strain 0346 these appendages have also been observed in ultrathin sections. Tweedy *et al.* (1968) predicted from shadowed preparations of *P. multivorans* NCIB 9691 that the diameter of the fimbriae of this strain in negative contrast preparations should be about 60 Å. The fimbrial diameter measurement for strain 0346 in this study (i.e. 62 ± 5 Å) is very close to that predicted by Tweedy *et al.* (1968).

The occurrence of polar fimbriae in three biotypes of *Pseudomonas solanacearum* is of interest; the only other plant pathogenic bacteria so far reported to possess fimbriae are some species of *Agrobacterium* (De Ley, Bernaerts, Rassel & Guilmot, 1966). The presence of polar fimbriae on cells of both *P. acidovorans* and *P. testosteroni* is also noteworthy, since these species are considered to be closely related (Stanier *et al.* 1966).

The fimbriae of *Pseudomonas testosteroni* and *P. fragi* appear to exhibit a periodic substructure suggesting that they are composed of repeating subunits of some kind. Regular periodic substructure has been observed before in the fimbriae of several bacteria (e.g. Thornley & Horne, 1962; Meynell & Lawn, 1967), including *P. echinoides* (Heumann & Marx, 1964). The appearance of the filaments of *P. testosteroni* seen in Pl. 1, fig. 2 is similar to that of F-actin filaments from muscle seen in negative contrast preparations (Hanson & Lowy, 1963; Haggis, 1966). The model of F-actin substructure constructed by Hanson & Lowy (1963) consisting of two helically wound strands composed of globular subunits might also apply to fimbriae of similar appearance.

Dark central regions, 13 to 19 Å wide, were seen in fimbriae of strain 0346 of *Pseudomonas multivorans* and *P. fragi* when these were prepared by negative contrast staining. Lawn (1966) has observed central dense lines of maximum diameter 15 Å in both common fimbriae and F-fimbriae of *Escherichia coli* when negatively stained. Brinton (1967) states that an axial hole 20 to 25 Å wide occurs in the Type 1 fimbriae of *E. coli* while the axial hole of F-fimbriae is 25 to 30 Å wide. The central regions seen in the fimbriae of *P. multivorans* and *P. fragi* in this study may indicate that the fimbriae possess axial holes along which negative contrast stain can penetrate, similar to those postulated to exist in Type 1 fimbriae and F-fimbriae of *E. coli*.

Present classifications of fimbriae are in our opinion artificial and premature. They have already given rise to confusion (Duguid & Anderson, 1967). No attempt has therefore been made to identify fimbriae of pseudomonads with any of the existing 'Types' in the existing classification schemes.

We wish to stress the possible value of the occurrence and distribution of fimbria-like appendages as taxonomic criteria. With the advent of improved negative contrast staining techniques and the more general use of electron microscopy, such features may be of use in the characterization and identification of bacteria. However, the reproducibility of electron microscopic results and the consistency of fimbriation throughout a particular taxonomic group must be considered. For instance, with respect to the former problem, it is important to know the conditions which are optimal for the production of fimbriae by pseudomonads. Studies on these aspects are proceeding in this laboratory.

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EXPLANATION OF PLATES

Abbreviations in figures: F, flagellum; P fimbria.

PLATE I

Fig. 1. *Pseudomonas aeruginosa* ATCC 14216 after 2×24 hr + 1×22 hr serial subcultures in lactate mineral salts medium incubated at 28° . At least two fimbriae arise from one of the cells. Negatively stained with sodium phosphotungstate, pH 7.4. $\times 160,000$.

Fig. 2. *P. testosteroni* ATCC 11996 after growth for 48 hr in peptone yeast extract broth incubated at 28° . Five fimbrial filaments are visible. Negatively stained with sodium phosphotungstate, pH 7.0. $\times 160,000$.

PLATE 2

Fig. 3. *P. acidovorans* ATCC 15668 after 1×48 hr + 1×72 hr + 1×88 hr serial subcultures in lactate mineral salts medium incubated at 28° . Two fimbriae are visible. Negatively stained with sodium phosphotungstate, pH 7.0. $\times 80,000$.

Fig. 4. *P. alcaligenes* ATCC 14909 after growth for 88 hr in peptone yeast extract broth incubated at 28° . One long fimbria is visible, folded across the flagellum in some parts. Negatively stained with sodium phosphotungstate, pH 7.0. $\times 80,000$.

Fig. 5. *P. multivorans* strain 0346 after 2×24 hr + 1×20 hr serial subcultures in lactate mineral salts medium incubated at 28° . Numerous peritrichous straight fimbriae are visible. Negatively stained with sodium phosphotungstate, pH 7.4. $\times 36,000$.

PLATE 3

Fig. 6. *P. multivorans* strain 0346 after 2×24 hr + 1×22 hr serial subcultures in lactate mineral salts medium incubated at 28° . Several fimbriae are visible, some of which display dark central regions. Negatively stained with sodium phosphotungstate. pH 7.4. $\times 160,000$.

Fig. 7. *P. multivorans* strain 0346 after growth for 22 hr in peptone yeast extract broth incubated at 28° . Numerous peritrichous appendages are visible. Shadowed with platinum/carbon. $\times 44,000$.

PLATE 4

Fig. 8. *P. multivorans* strain 0346 after growth for 26 hr in lactate mineral salts medium incubated at 28° . Numerous filamentous appendages extend from the periphery of sectioned cells. Glutaraldehyde-osmium tetroxide fixation. $\times 128,000$.

Fig. 9. *P. fragi* ATCC 4973 after 1×24 hr + 1×39 hr serial subcultures in lactate mineral salts medium. Numerous peritrichous fimbriae are visible. Negatively stained with sodium phosphotungstate, pH 7.0. $\times 52,000$.

PLATE 5

Fig. 10. *P. fragi* ATCC 4973 after 2×24 hr + 1×22 hr serial subcultures in lactate mineral salts medium at 28° . Straight fimbriae displaying dark central regions are visible. Negatively stained with sodium phosphotungstate, pH 7.0. $\times 272,000$.

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Fig. 11. *P. maltophilia* strain 0147 after 1×48 hr + 1×72 hr + 1×88 hr serial subcultures in lactate mineral salts medium containing methionine, incubated at 28° . Two curved fimbriae are visible. Negatively stained with sodium phosphotungstate, pH 7.0. $\times 80,000$.

PLATE 6

Fig. 12. *P. solanacearum* strain 017 A, biotype II after growth for 64 hr in sucrose peptone broth incubated at 28° . Three fimbriae extend from a flagellated cell. Negatively stained with sodium phosphotungstate, pH 6.0. $\times 88,000$.

Fig. 13. *P. solanacearum* strain 002 A, biotype III after growth for 48 hr in sucrose peptone broth incubated at 28° . Six fimbriae extend from one pole of the cell. Negatively stained with sodium phosphotungstate, pH 7.0. $\times 40,000$.