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Production of Pili (Fimbriae) by *Pseudomonas fluorescens* and Correlation with Attachment to Corn Roots†

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***Pseudomonas fluorescens* isolates 13525 and 2-79 were grown in Luria broth and low-nutrient medium (LNM). Pililike fibrils were very rarely produced in Luria broth but were abundantly produced in LNM. In LNM the pili were peritrichously distributed and had diameters ranging from 3 to 8 nm. Pili were purified from strain 2-79, and the pilin subunit was found to have a molecular weight of about 34,000. Strain 2-79 produced two colony types on Luria agar, nonmucoidal and mucoidal. Cells in LNM cultures of the nonmucoidal colony type were highly piliated, and cells from the mucoidal type were nearly devoid of pili. The presence of pili on nonmucoidal isolate 2-79 was quantitatively correlated with hydrophobic attachment to polystyrene, hemagglutination, and attachment to corn roots.**

Fluorescent pseudomonads are commonly isolated from the plant rhizosphere (5, 33). Some strains have been shown to protect plants from fungal diseases (16, 17, 21, 33, 46). Two factors have been cited as being essential to this biocontrol: colonization of the rhizosphere and production of antifungal substances (33). Although much has been learned about the antifungal substances (32), little is known about the colonization of roots. Many factors probably contribute to colonization of plant roots by bacteria including motility, chemotaxis, carbohydrate utilization, and attachment. Little is known about the mechanism(s) of initial attachment of fluorescent pseudomonads to root surfaces. In this report the possibility of pili (fimbriae) as being one mechanism of *Pseudomonas fluorescens* attachment to corn roots is examined.

Pili (fimbriae) are proteinaceous hairlike structures that are produced most commonly by gram-negative bacteria (29). One function associated with fimbriae is attachment or adhesion to a wide variety of organisms and substrates (18). Many animal pathogens attach to their hosts by means of fimbriae, and mutants that do not produce fimbriae neither attach nor cause disease (18).

Evidence for the pili-mediated attachment of bacteria to plants is accumulating. Duguid (7) noted that saprophytic *Klebsiella* strains adhere, apparently by their fimbriae, to the root hairs of seedlings. It has now been demonstrated that pili mediate the attachment of *Klebsiella pneumoniae* and *Enterobacter agglomerans* to *Poa pratensis* roots (14, 22, 24). In addition, Vesper and Bauer (43) found that the firm attachment of *Rhizobium japonicum* to soybean roots is mediated by pili with a subunit molecular weight of 21,000. It also appears that the attachment to pea roots by *Rhizobium leguminosarum* may be mediated by fimbriae (37).

Little is known about the mechanism of *Pseudomonas fluorescens* attachment to plant roots. Fuerst and Hayward (12) examined 15 species of pseudomonads and found 8 that produced fimbriae. Their limited survey of *P. fluorescens* strains did not reveal the presence of fimbriae, although they pointed out that the test conditions may not have been right for the expression of fimbriae.

Pseudomonas aeruginosa produces pili which mediate the attachment to animal tissues (47). It appears that *Pseudomonas solanacerarum* attaches by fimbriae to cultured plant cells (38). There are several observations which suggest that fimbriae may be involved in the attachment of other pseudomonads. *Pseudomonas eichinoides* produces fimbriae which cause hemagglutination, but their role in attachment to other organisms was not tested (15). *Pseudomonas tolaasii* was observed to attach to mushroom mycelia by rodlike strucutres (30), the nature of which was not examined.

In this report is presented the first evidence for the occurrence of pili (fimbriae) on *P. fluorescens* strains, and it is suggested that their occurrence is correlated with the ability of this bacterium to attach to corn roots.

MATERIALS AND METHODS

***P. fluorescens* strains and sources.** *P. fluorescens* isolate 2-79 was obtained from D. Weller (Department of Plant Pathology, Washington State University, Pullman, Wash.). *P. fluorescens* 13525 is the type culture for *P. fluorescens* species and was obtained from the American Type Culture Collection (Rockville, Md.). Strains were stored in water as described previously (6).

Media. Luria broth (LB) was prepared as described by Maniatis et al. (27). The low-nutrient medium (LNM) contained the following per liter: $(NH_4)_2SO_4$, 1.32 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; K_2HPO_4, 0.5 g; NaCl, 0.1 g; yeast extract, 0.1 g; succinic acid, 1.5 g. The pH was adjusted to 6.8. Solidified media were produced by the addition of 15 g of agar per liter.

Attachment to corn roots. Corn seeds (*Zea mays* cv. C744X) were obtained from CountryMark (Springfield, Ohio) and surface sterilized for 15 min in 5.25% sodium hypochlorite and then rinsed in sterile distilled water. The seeds were allowed to soak for 1 to 2 h and then placed in sterile stainless steel serving pans (24 by 29 by 5 cm; catalog no. 9471-N42; Thomas Scientific), containing two sheets of Whatman no. 1 filter paper cut to fit the bottom of the pan and 50 ml of sterile, distilled water. After 3 days of growth at 27°C, the roots were used in the attachment assay.

Attachment assay. The attachment assay was performed as described previously (42) with the following modifications. The terminal 1 cm of each root was used. The bacterial cells were suspended in sterile, distilled water from colonies that had been growing for 14 to 16 h on L-agar plates to give an

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A_{620} of 0.2. This suspension was diluted 1:100 in Hoagland solution, giving a final concentration of approximately 1×10^6 to 5×10^6 bacteria per ml. The exact initial bacterial concentration was determined by serial dilution plating of samples from this bacterial suspension on L-agar. Sets of 10 roots were added to culture dishes (80 by 110 mm; Pyrex; Corning Glass Works, Corning, N.Y.) containing 100 ml of the bacterial suspension. The suspensions were greatly agitated during incubation on a rotary shaker at 50 rpm. After the roots were exposed to the bacterial suspension for 30 min, the root segments were gently removed from the dish with forceps, drained for a few seconds on sterile filter paper, transferred with forceps to 700 ml of sterile Hoagland solution, and vigorously shaken for 10 s to remove the unattached bacteria. The root segments were recovered on a Büchner funnel and placed in 10-ml test tube containing 2 ml of water plus Tween 40 (1 drop in 100 ml of water). The tubes were placed on the cup-horn unit of a sonicator (model 370; Heat Systems). The attached bacterial cells were released by sonication for 4 min at 50% power. (In preliminary tests, comparable sets of roots were homogenized, and the homogenate was grown on agar plates to recover the attached population.) Fractions of the suspension of released bacteria were then plated directly, or diluted and plated, onto L-agar with a plater (model DU; Spiral System Inc., Cincinnati, Ohio).

Because the initial populations varied slightly in different experiments and in different suspensions, the results are expressed as relative attachment. The size of the original bacterial population was adjusted or normalized to 10^6 cells per ml, and the attached population was adjusted as a function of the difference in the size of the actual population from that of the normalized population.

Hemagglutination. Bovine (ox), chicken, human (type A), rabbit, and horse (Sigma Chemical Co., St. Louis, Mo.) erythrocytes were reconstituted in phosphate-buffered saline (PBS) by the instructions of the manufacturer. Mucoidal and nonmucoidal cultures of *P. fluorescens* 2-79 were grown in LNM overnight. Hemagglutination tests were performed on glass microscope slides by mixing approximately 50 μl (1 drop) of the culture with approximately 50 μl (1 drop) of blood or PBS (as the control) with a sterile toothpick and by then gently rocking for 1 to 2 min. Hemagglutination was rated visually.

Possible sugar inhibition of hemagglutination was tested by making 0.5 M solutions of D-glucose, D-galactose, D-mannose, L-fucose, L-rhamnose, D-xylose, D-galacturonic acid, and D-glucuronic acid (all from Sigma) and L-arabinose (Calbiochem-Behring, La Jolla, Calif.) in PBS. Three serial twofold dilutions were also prepared from each solution in PBS. About 50 μl (1 drop) of an overnight culture of nonmucoidal strain 2-79 in LNM was mixed with about 50 μl (1 drop) of each of the sugar solutions (1 drop of PBS as a control), and the mixtures were gently mixed on a rotary shaker for 1 h. The hemagglutination assay then was carried out as described above. The level of inhibition was rated visually.

Piliated cell population estimates. The number of cells with pili was estimated by using two techniques, direct counts by transmission electron microscopy (TEM) and hydrophobic attachment to polystyrene. The TEM counts were made as described previously (43) with the modification that a 1% uranyl acetate solution was used for negative staining. Observations were made with an electron microscope (model 200; Philips).

The estimates of hydrophobicity were made as described previously (43), with the modification that the populations tested were those produced by resuspension in sterile water of colonies grown on L-agar plates.

The hydrophobicity was estimated from the bacterial resuspension (A_{620}, 0.2) by placing 5 ml of the suspended cells in three glass and three plastic petri dishes. The suspension was gently shaken so that the liquid spread over the bottom of each dish. The plates were placed in a cold room for 1 h. Then the plates were allowed to come to room temperature. The plates were gently rocked to suspend settled but not attached cells. The optical density of each suspension was read at 620 nm, and the difference between the optical density of the suspension from the glass and the plastic petri dishes was determined. The hydrophobic attachment is expressed as a percentage change in the free population reflected in the change in optical density due to binding to the hydrophobic polystyrene. The results are based on three replicate plates and three separate experiments, and the values are the averages ± standard deviations.

Purification of pili. Pili were isolated from a nonmucoidal form of *P. fluorescens* 2-79 grown on a rotary shaker at 100 rpm and 28°C in 2.8-liter Fernbach flasks containing 2 liters of LNM. Cells were harvested after 24 h of growth by centrifugation at 9,000 × *g* for 10 min. The pellets were suspended in water and blended in an homogenizer (model 45; The VirTis Co., Inc., Gardiner, N.Y.) at a setting of 30 for 5 min and then centrifuged at 10,000 × *g* for 15 min. Pili contained in the supernatant were purified as described by Korhonen et al. (23). Pili were precipitated with ammonium sulfate, solubilized with sodium deoxycholate, fractionated by sucrose density gradient centrifugation, and further purified by gel filtration on Sepharose 4B.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed in 1-mm slab gels containing 12.6% acrylamide by using a modification of the method described by Laemmli (25). The marker proteins used were pepsin (porcine stomach mucosa), trypsinogen (bovine stomach mucosa), and β-lactoglobulin (bovine milk; Sigma). To break the pilus complexes into subunits, the purified pili were placed in sample buffer and vortexed at a setting of 4 on a Vortex-Genie (Scientific Instruments, Springfield, Mass.) for 8 h before they were boiled and applied to the gel. The gels were stained with silver nitrate (48).

RESULTS

Growth of *P. fluorescens* strains. *P. fluorescens* strains were initially grown in LB. Two colony forms of isolate 2-79 were discovered when it was grown on L-agar, and these were designated mucoidal and nonmucoidal (Fig. 1). Pilus-like fibrils were occasionally seen on cells of *P. fluorescens* strains grown in LB medium, but they were rare and few in number on those cells that produced them. Alternative media were sought to enhance the production of these fibrils. The LNM was devised. When the various strains were grown in LNM, pili were produced in abundance on strain 13525 and the nonmucoidal form of 2-79 (Table 1 and Fig. 2A and B) but were uncommon on the mucoidal form of 2-79 (Table 1). The pili were peritrichously distributed. At high magnification the typically helical arrangement of pilin subunits was apparent (Fig. 3). This is the first report of these structures being produced by *P. fluorescens*.

The relationship between mucoidal and nonmucoidal 2-79 isolates was examined. Mucoidal or nonmucoidal colonies produced after about 16 h of growth on L-agar, when

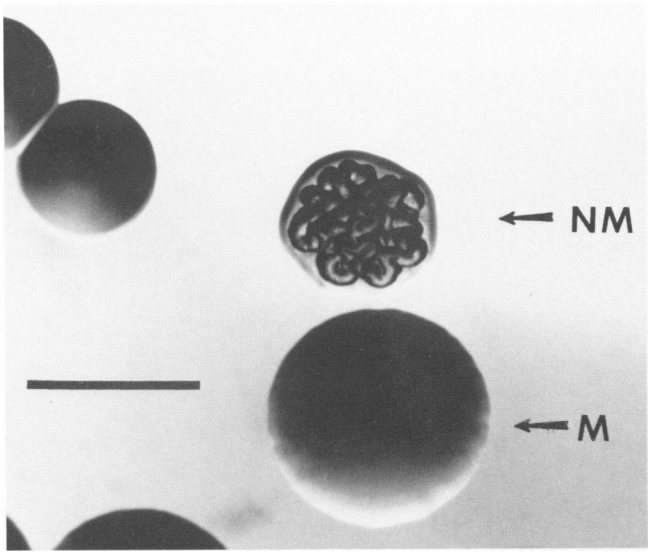

FIG. 1. Two colony types of *P. fluorescens* isolate 2-79 produced on LB agar at about 40 h. M is mucoidal and NM is nonmucoidal. Bar, 1 mm.

restreaked, always gave rise to colonies of the same type. On the second day (40 h) of growth on L-agar, however, all nonmucoidal colonies began to show a ring around the edge which expanded with further growth (Fig. 4). The mucoidal colonies maintained their basic appearance with continued growth. If an isolation was made from this outer ring of older nonmucoidal colonies, a pure isolation of mucoidal colonies resulted. However, when 2-79 mucoidal and nonmucoidal colonies were grown on LNM agar, all colonies appeared to be about the same (Fig. 5). Yet, when colonies growing on LNM for 2 weeks were restreaked onto L-agar, the mucoidal colonies gave rise to pure mucoidal colonies and nonmucoidal colonies gave rise to pure nonmucoidal colonies. Strain 13525 produced no discernibly different colony types on L-agar or LNM agar plates.

Correlation between attachment, piliation, and hydrophobicity. The original source of isolate 2-79 consisted of about 98% mucoidal colonies. In early attachment studies, however, it was discovered that the nonmucoidal colony was disproportionately represened on the root surface, making up to 60% of the attached cells. Because of the correlation between colony morphology and piliation, the attachment level to corn roots for each different form of 2-79 was tested. When the 2-79 nonmucoidal isolate was grown in LNM broth, it formed such massive clumps that it was impossible to treat it as a uniformly dispersed population. The mucoidal 2-79 isolate formed a nonclumped culture.

In preliminary tests, the use of sonication to release the attached bacteria was found to be just as effective as homogenization to enumerate the attached bacteria (data not shown). Because sonication is simpler and more efficient to perform, it was the method of choice for these experiments. Also, results of preliminary time course studies indicated that attachment to corn roots is a linear function with time after the first few minutes until at least 2 h of exposure (data not shown). Therefore, the 30-min time point for these studies was chosen as representative of attachment.

To obtain nonclumping or low-clumping populations, *P. fluorescens* isolate 2-79 mucoidal and nonmucoidal colonies were grown on L-agar plates for 14 to 16 h (before the switch to a mucoidal colony was apparent around the nonmucoidal colony) and suspended. These suspended cells were tested for the level of piliation, their ability to attach to corn roots, and their ability to bind to polystyrene (Table 2). There was a clear trend showing a correlation between the level of piliation, the ability to attach to polystyrene, and the ability to bind to corn roots.

Hemagglutination by *P. fluorescens* strains. Hemagglutination ability is often correlated with pilus-mediated binding. Therefore, a variety of erythrocytes was tested for hemagglutination by mucoidal and nonmucoidal cultures. The nonmucoidal form of isolate 2-79 readily hemagglutinated all the blood types tested (bovine, chicken, human, rabbit, and horse), but the mucoidal form failed to hemagglutinate any of the various blood types (Table 3). The hemagglutination by isolate 13525 varied only slightly from that of nonmucoidal isolate 2-79 (Table 3).

Most of the common plant cell wall sugars that were tested failed to inhibit the hemagglutination of bovine erythrocytes by nonmucoidal 2-79 isolates (Table 4). D-Galactose and D-galacturonic acid at 0.5 and 0.25 M concentrations, however, partially inhibited this hemagglutination.

Isolation and purification of *P. fluorescens* 2-79 pili. Pili were isolated from nonmucoidal *P. fluorescens* 2-79. Twenty grams (fresh weight) of *P. fluorescens* 2–79 cells grown in LNM yielded about 1 mg of purified pilin protein. The pili banded at the 39 to 43% sucrose level in a sucrose density gradient. Massive ropelike reaggregations formed after final dialysis of the gel filtration fraction (Fig. 6). Vigorous vortexing was necessary to get the pilin subunits disaggregated. Two protein bands were observed after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 7). The major pilin subunit has a molecular weight of about 34,000 and a smaller band occurs at about 28,000.

DISCUSSION

The fibrillar structures on *P. fluorescens* can be identified as pili (fimbriae) because of the many characteristics they have in common with other bacterial pili. The pili produced by *P. fluorescens* isolates 13525 and 2-79 varied in diameter from 3 to 8 nm. This is a diameter range that is consistent with most pili (18). The pili are basically straight, nonsinusoidal structures that are clearly distinct from flagella (29). The helical arrangement of the pilin subunits in *P. fluorescens* 2-79 pili is consistent with the reported structure of other pili (39, 45). Peritrichous distribution of pili on bacterial surfaces is most common on gram-negative bacteria (29), but both polar and peritrichously distributed pili have been described on various species of *Pseudomonas* (12).

TABLE 1. Population, diameter, and number of pili on *P. fluorescens* strains grown in shake cultures of LNM[a]

Strain	% Piliated[b]	Diameter (nm)[c]	No. of pili/cell[d]
2-79			
Nonmucoidal	60 ± 14	7–8	Up to 200
Mucoidal	1 ± 1	ND[e]	ND
13525	25 ± 5	3–4	Up to 300

[a] Strains were grown overnight in LNM medium, placed on Formvar-coated grids, stained with uranyl acetate, and examined by TEM.
[b] The number of piliated cells was based on random counts of 100 cells. The values given are the averages of three replicates ± standard deviation.
[c] The diameters are based on direct measurements from TEM negatives.
[d] The number of pili per cell was estimated from printed micrographs.
[e] ND, Not determined.

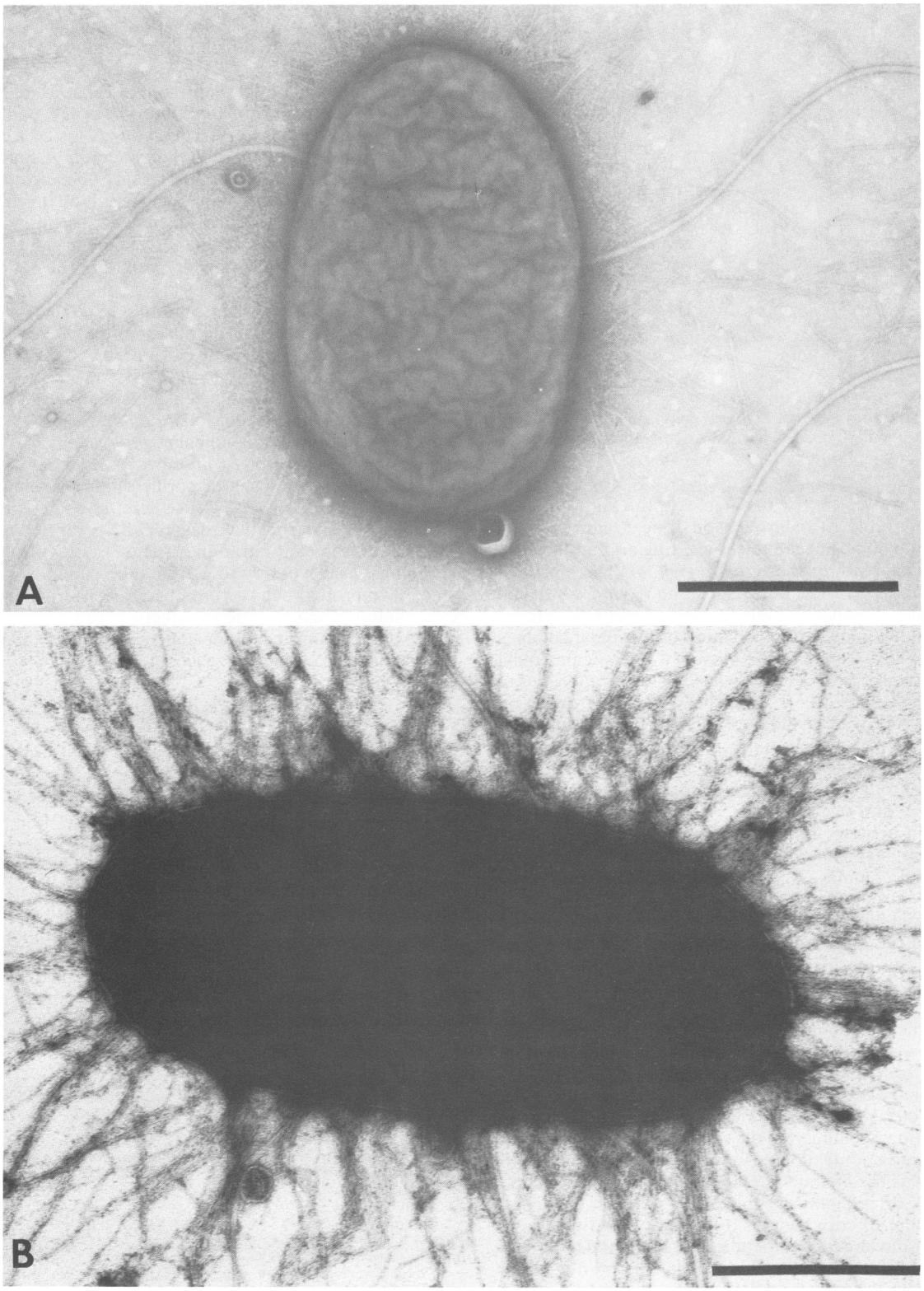

FIG. 2. Appearance of a piliated cell from overnight cultures of nonmucoidal *P. fluorescens* isolate, 2-79 (A) and 13525 (B) grown in LNM. Bars, 0.5 μm.

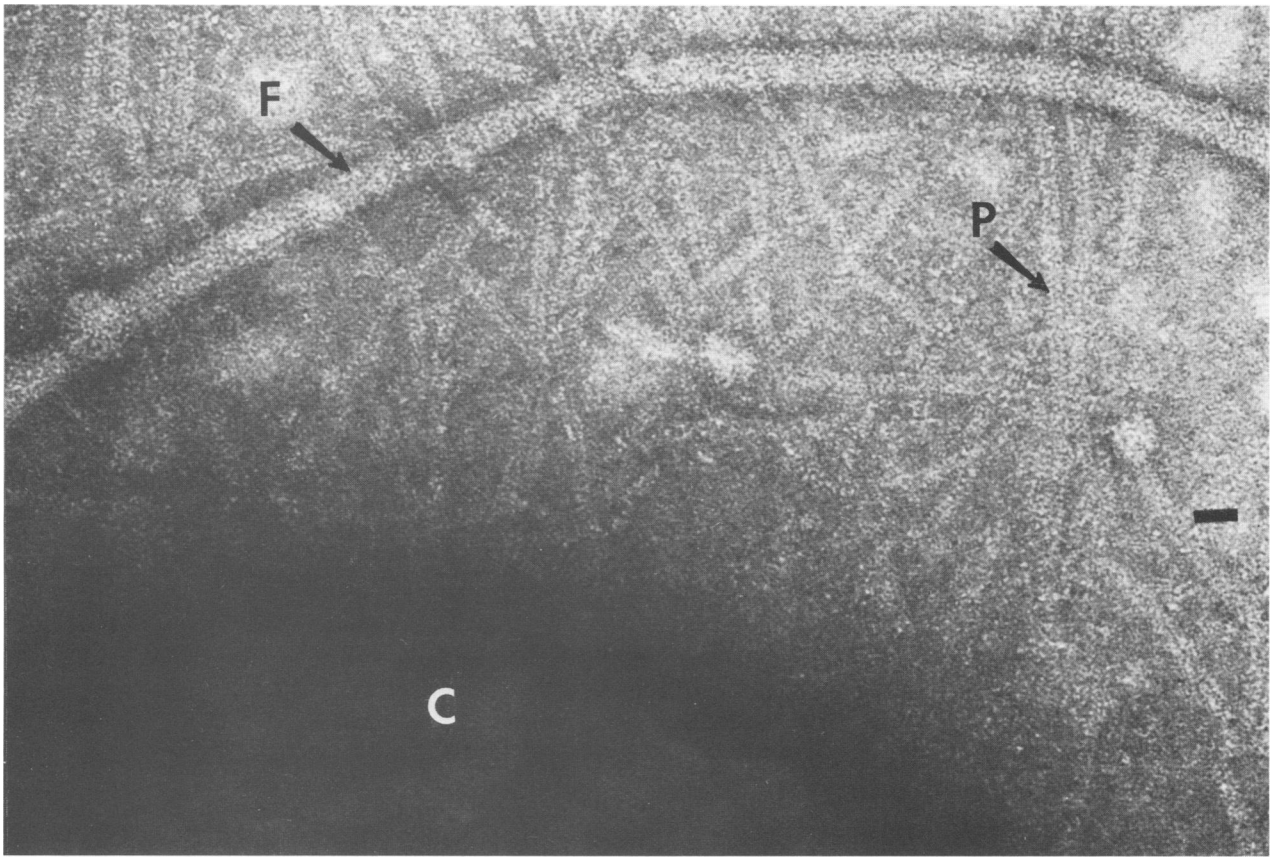

FIG. 3. Helical arrangement of the pilin subunits of *P. fluorescens* nonmucoidal isolate 2-79 when grown in LNM. Magnification, approximately ×150,000. Bar, 10 nm. Abbreviations: C, cell; P, pilus; F, flagellum.

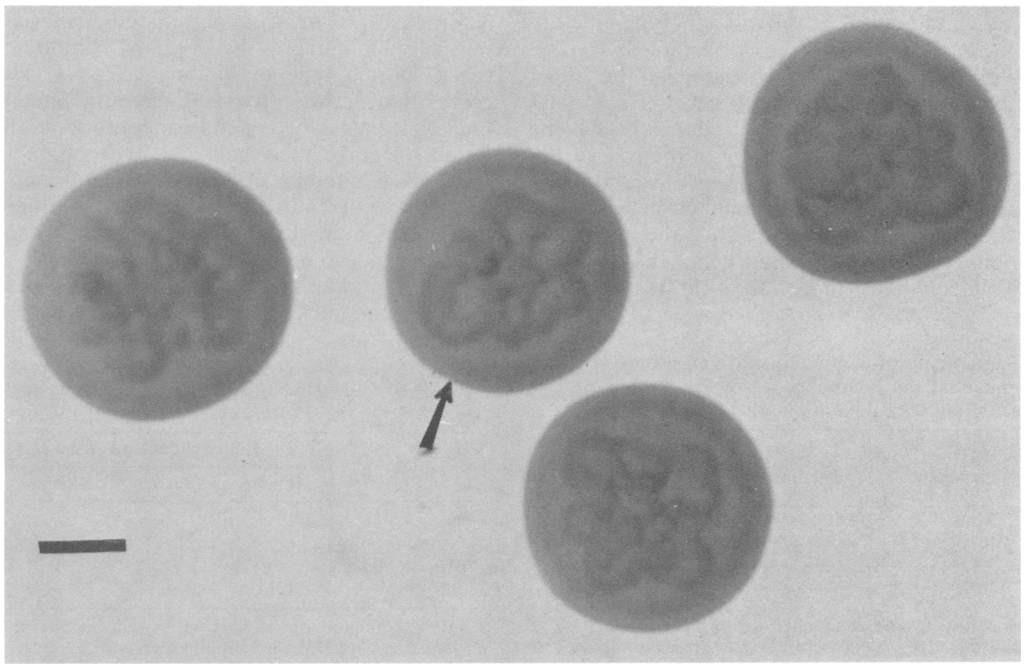

FIG. 4. Appearance of nonmucoidal *P. fluorescens* isolate 2-79 colonies after 4 days of growth on Luria agar. Arrow indicates the outer mucoidal ring. Bar, 1 mm.

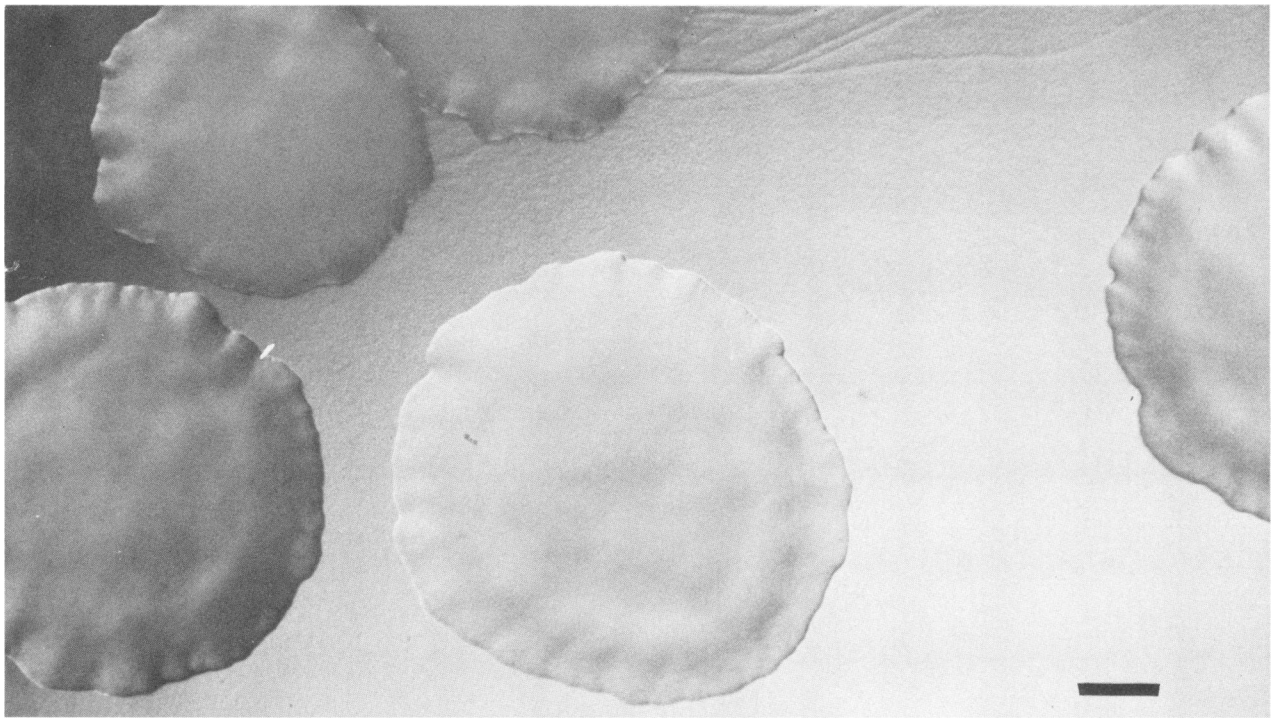

FIG. 5. Appearance of mucoidal or nonmucoidal colonies of isolate 2-79 when grown on LNM agar for 7 days. Bar, 1 mm.

The molecular weight of *P. fluorescens* pilin is rather large (34,000). However, pilin subunits from 8,000 to 64,000 daltons have been reported (18). Massive self-reassembly of *P. fluorescens* 2-79 pilin subunits into long ropelike masses apparently occurs has been as described previously for *P. solancearum* pili (49). A second minor band was also obtained in the purified sample of *P. fluorescens* pili. This band may represent a second type of pilus which was not visibly recognized. It is also possible that the minor band represents a protease cleavage product (11) of the major polypeptide or an artifact of the reaction with mercaptoethanol (20). The rather vigorous efforts required to disaggregate the pilin complexes may have induced such an artifact.

The occurrence of a natural variant of isolate 2-79, which was highly piliated, allowed for the testing of the role of *P. fluorescens* pili in attachment to roots. There was a good correlation between the occurrence of piliated cells in the population and the level of attachment. While the mucoidal colony type produced very few pilated cells, the nonmucoidal type had substantially more. It is interesting that although the nonmucoidal colony type was associated with pili production, not all cells produced pili, at least not at the same time. The increased level of attachment to corn roots by the nonmucoidal form of *P. fluorescens* correlated with the increased level of piliation.

Two other correlations relate piliation in the nonmucoidal colony to attachment. These were the level of binding to polystyrene and hemagglutination ability. Binding to hydrophobic substances or substrates has been shown as a characteristic of a number of piliated bacteria (31, 43). The normally high level of hydrophobic amino acids in pili may result in this type of binding (20, 44). There is a clear correlation between hydrophobic attachment by the highly piliated isolate 2-79 and attachment to roots. James et al. (19) found no correlation between hydrophobicity and attachment of a number of bacteria, including a strain of *P. fluorescens*, to radish roots. Under the different test conditions used here, hydrophobicity was a good indicator of piliation and attachment by *P. fluorescens* 2-79, but other strains should be tested to confirm this observation.

Many examples can be found of the correlation between

TABLE 2. Comparison of the attachment to corn by *P. fluorescens* 2-79 mucoidal and nonmucoidal colonies grown on L-agar and correlation with piliation and hydrophobicity

Isolate 2-79	Relative attachment[a]	% Piliation[b]	Hydrophobicity (% change in optical density)[c]
Mucoidal	1	0.3 ± 1	−3 ± 2
Nonmucoidal	11 ± 2	19 ± 1	15 ± 3

[a] See text for a description of the methods used to determine relative attachment.

[b] The number of piliated cells was determined for each experiment by random counts of 100 cells in the resuspension. The values given are averages for three replicate counts ± standard deviation.

[c] See text for a description of the methods used to determine hydrophobicity.

TABLE 3. Hemagglutination of various erythrocytes by *P. fluorescens* 2-79 and 13525[a]

Strain	Hemagglutination of the erythrocytes from[b]				
	Bovine	Chicken	Human	Rabbit	Horse
2-79					
Nonmucoidal	+++	+++	+++	++	++
Mucoidal	−	−	−	−	−
13525	ND[c]	+++	++	++	+++

[a] Hemagglutination tests were performed as described in the text. Overnight cultures in LNM were used in each case.

[b] Each hemagglutination reaction was rated visually, with +++ being complete agglutination and − being no agglutination.

[c] ND, Not determined.

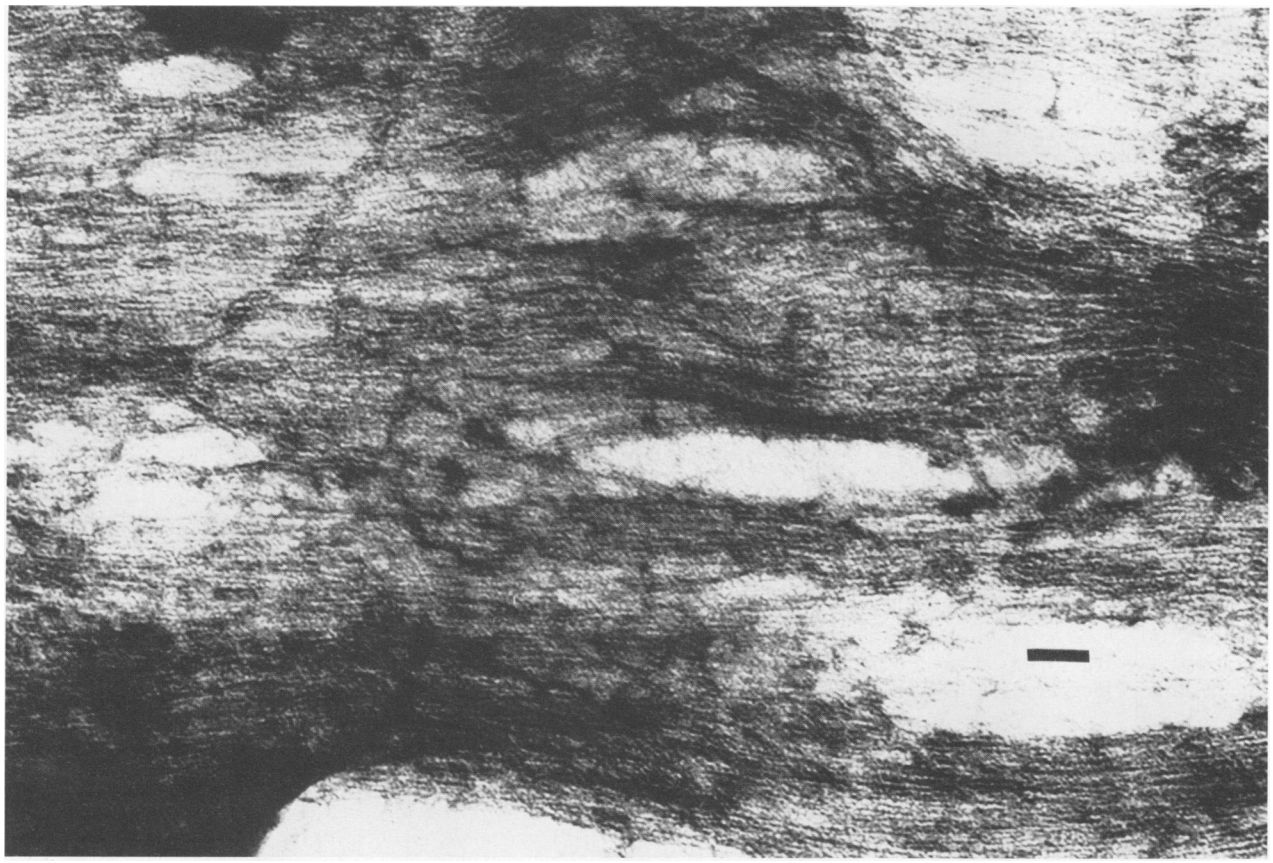

FIG. 6. Transmission electron micrograph of purified pili aggregates formed after the dialysis of the gel filtration fractions. Bar, 0.1 μm.

bacterial hemagglutination and the occurrence of pili on bacterial surfaces (1, 4, 8, 10, 26, 34). There is usually a specificity in the inhibition of hemagglutination by certain carbohydrates (18). The carbohydrate inhibitors of hemagglutination can often reveal information about the nature of the pilus binding sites. Gilboa-Garber (13) described a D-galactose-sensitive hemagglutinin from *P. aeruginosa*. In a survey of nearly 400 strains of soil bacteria, about 10% were found to be active in hemagglutination (40). All the hemagglutinins of these soil bacteria, most of which were pseudomonads, were D-galactose- or *N*-acetyl-D-galactosamine sensitive (40). The highly piliated *P. fluorescens* isolate 2-79 was found to hemagglutinate five blood cell types very rapidly (less than 2 min). The mucoidal form did not agglutinate any of the blood types under the same conditions. The correlation between pili and hemagglutination is consistent with what is known about the functions of pili.

TABLE 4. Inhibition of nonmucoidal *P. fluorescens* 2-79 hemagglutination by plant root sugars

Sugar[a]	Inhibition at the following dilutions[b]:			
	1	2	3	4
L-Rhamnose	+++	+++	+++	+++
D-Galacturonic acid	+	++	+++	+++
L-Arabinose	+++	+++	+++	+++
D-Glucose	+++	+++	+++	+++
D-Galactose	+	++	+++	+++
D-Mannose	+++	+++	+++	+++
D-Glucuronic acid	+++	+++	+++	+++
D-Xylose	+++	+++	+++	+++
L-Fucose	+++	+++	+++	+++

[a] Sugar solutions were prepared as described in the text.

[b] Dilution 1 was 0.5 M, with each dilution being a twofold dilution in PBS of the one before. Each hemagglutination reaction was rated visually, with +++ being complete agglutination and − being no agglutination. All of the highest sugar concentration agglutination tests were repeated at least three times.

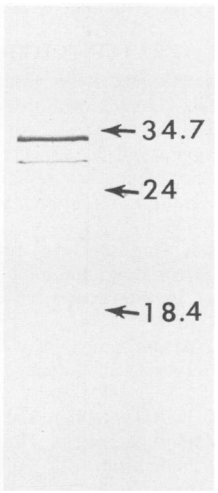

FIG. 7. Molecular weight determination of pilin protein isolated from nonmucoidal *P. fluorescens* isolate 2-79. The standard proteins used were pepsin (molecular weight, 34,700), trypsinogen (molecular weight, 24,000), and β-lactoglobulin (molecular weight, 18,400).

The partial inhibition of hemagglutination by galactose and galacturonic acid may suggest possible sites of pilus attachment to root surfaces. Rather high concentrations (0.5 to 0.25 M) of the carbohydrates were required to inhibit hemagglutination, however. It may be that galactose and galacturonic acid are only a portion of the active binding site.

The expression of pili on bacteria has been divided into two general processes: a qualitative variation called phase variation and a second process called quantitative variation, which governs the amount of pili produced (18). Phase variation is usually a pilus on or off condition and is often apparent from some colony morphology change (2, 3, 35, 41). The evidence presented here strongly suggests that phase variation is occurring in *P. fluorescens* 2-79. It is clear that a change in colony morphology occurs. This is only obvious on L-agar, however, it appears that growth of nonmucoidal 2-79 isolates on LNM agar somehow inhibits the switch to the less-piliated mucoidal form.

Phase variation of piliated bacteria has been examined in a number of strains. It now appears that an on-off switch which is dependent on the inversion of a small DNA sequence may control phase variation. The work of Eisenstein (9) and Meyer et al. (28) supports this kind of control mechanism for *Escherichia coli* and *Neisseria gonorrhoeae* pili, respectively. This form of regulation may be similar to the mechanism which controls flagellar type in *Salmonella* species (36).

Production of most pili is dependent on quantitative variation, which is often controlled by some environmental factor (18). There is marked difference in the production of pili by nonmucoidal *P. fluorescens* isolate 2-79 in LB and LNM broth. In the case of *P. fluorescens* isolate 13525 and nonmucoidal isolate 2-79, it is likely that some medium component affects the quantitative expression of pili. Interestingly, mucoidal 2-79 does not appear to be affected by the same medium component. Much remains to be learned about LNM and its affect on the level of piliation. Ultimately, it remains to be seen how *P. fluorescens* growth in soil and in the rhizosphere affects piliation and attachment.

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LITERATURE CITED

1. **Arai, H., and J. J. Munoz.** 1979. Fimbrial hemagglutinin in stationary and shake cultures of *Bordetella pertussis*. Infect. Immun. **25**:764–767.
2. **Bovre, K., T. Bergan, and L. O. Froholm.** 1970. Electron microscopical and serological characteristics associated with colony type in *Moraxella nonliquefaciens*. Acta Pathol. Microbiol. Scand. **78**:649–659.
3. **Brinton, C. C.** 1965. The structure, function, synthesis, and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram negative bacteria. Trans. N.Y. Acad. Sci. **27**:1003–1054.
4. **Christofi, N., M. I. Wilson, and D. C. Old.** 1979. Fimbriae and haemagglutinins in erwinias of the *Carotovora* group. J. Appl. Bacteriol. **46**:179–183.
5. **Cook, J. R., and K. F. Baker.** 1983. The nature and practice of biological control of plant pathogens. The American Phytopathological Society, St. Paul, Minn.
6. **Crist, D. K., R. E. Wyza, K. K. Mills, W. D. Bauer, and W. R. Evans.** 1984. Preservation of *Rhizobium* viability and symbiotic infectivity by suspension in water. Appl. Environ. Microbiol. **47**:895–900.
7. **Duguid, J. P.** 1959. Fimbriae and adhesive properties in *Klebsiella* strains. J. Gen. Microbiol. **21**:271–286.
8. **Duguid, J. P., I. W. Smith, G. Dempster, and P. N. Edmunds.** 1955. Non-flagellar filamentous appendages ("fimbriae") and haemagglutinating activity in *Bacterium coli*. J. Pathol. Bacteriol. **70**:335–348.
9. **Eisenstein, B. I.** 1981. Phase variation of type I fimbriae in *Escherichia coli* is under transcriptional control. Science **214**: 337–339.
10. **Evans, D. J., Jr., D. G. Evans, and H. L. DePont.** 1979. Hemagglutination patterns of enterotoxigenic and enteropathogenic *Escherichia coli* determined with human, bovine, chicken, and guinea pig erythrocytes in the presence and absence of mannose. Infect. Immun. **23**:336–346.
11. **Fader, R. C., L. K. Duffy, C. P. Davis, and A. Kurosky.** 1982. Purification and chemical characterization of type I pili isolated from *Klebsiella pneumoniae*. J. Biol. Chem. **257**:3301–3305.
12. **Fuerst, J. A., and A. C. Hayward.** 1969. Surface appendages similar to fimbriae (pili) on *Pseudomonas* species. J. Gen. Microbiol. **58**:227–237.
13. **Gilboa-Garber, N.** 1972. Inhibition of broad spectrum hemagglutinin from *Pseudomonas aeruginosa* by D-galactose and its derivatives. FEBS Lett. **20**:242–244.
14. **Haahtela, K., E. Tarkka, and T. K. Korhonen.** 1985. Type 1 fimbria-mediated adhesion of enteric bacteria to grass roots. Appl. Environ. Microbiol. **49**:1182–1185.
15. **Heumann, W., and R. Marx.** 1964. Feinstruktur und Funktion der Fimbrien bei dem sternbildenden Bakterium *Pseudomonas echinoides*. Arch. Mikrobiol. **47**:325–337.
16. **Howell, C. R., and R. D. Stipanovic.** 1978. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology **69**:480–482.
17. **Howell, C. R., and R. D. Stipanovic.** 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology **70**:712–715.
18. **Isaacson, R. E.** 1985. Pilus adhesions, p. 307–336. *In* D. C. Savage and M. Fletcher (ed.), Bacterial adhesion. Plenum Publishing Corp., New York.
19. **James, D. W., Jr., T. V. Suslow, and K. E. Steinback.** 1985. Relationship between rapid, firm adhesion and long-term colonization of roots by bacteria. Appl. Environ. Microbiol. **50**:392–397.
20. **Jones, G. W., and R. E. Isaacson.** 1982. Proteinaceous bacterial adhesins and their receptors. Crit. Rev. Microbiol. **10**:229–260.
21. **Kloepper, J. W., J. Leong, M. Teintze, and M. N. Schroth.** 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature (London) **286**:885–886.
22. **Korhonen, T. K., E.-L. Nurmiaho-Lassila, T. Laakso, and K. Haahtela.** 1986. Adhesion of fimbriated nitrogen-fixing enteric bacteria to roots of grasses and cereals. Plant Soil **90**:59–69.
23. **Korhonen, T. K., E. Nurmiaho, H. Ranta, and C. S. Ewden.** 1980. New method for isolation of immunologically pure pili from *Escherichia coli*. Infect. Immun. **27**:569–575.
24. **Korhonen, T. K., E. Tarkka, H. Ranta, and K. Haahtela.** 1983. Type 3 fimbriae of *Klebsiella* sp.: molecular characterization and role in bacterial adhesion to plant roots. J. Bacteriol. **155**:860–865.
25. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
26. **Maclagan, R. M., and D. C. Old.** 1980. Haemagglutinins and fimbriae in different serotypes and biotypes of *Yersinia enterocolitica*. J. Appl. Bacteriol. **49**:353–360.
27. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Meyer, T. F., N. Mlawer, and M. So.** 1982. Pilus expression in *Neisseria gonorrhoeae* involves chromosomal rearrangement. Cell **30**:45–52.
29. **Ottow, J. C. G.** 1975. Ecology, physiology and genetics of fimbriae and pili. Annu. Rev. Microbiol. **29**:79–108.

30. **Preece, T. F., and W. C. Wong.** 1982. Quantitative and scanning electron microscope observations on the attachment of *Pseudomonas tolaasii* and other bacteria to the surface of *Agaricus bisporus*. Physiol. Plant Pathol. **21**:251–257.
31. **Rosenberg, M., E. A. Bayer, J. Delarea, and E. Rosenberg.** 1982. Role of thin fimbriae in adherence and growth of *Acinetobacter calcoaceticus* RAG-1 on hexadecane. J. Bacteriol. **44**:929–937.
32. **Schroth, M. N., and J. G. Hancock.** 1982. Disease-suppressive soil and root-colonizing bacteria. Science **216**:1376–1381.
33. **Schroth, M. N., J. E. Loper, and D. C. Hildebrand.** 1984. Bacteria as biocontrol agents of plant disease, p. 362–369. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.
34. **Scott, S. S., and D. C. Old.** 1981. Mannose-resistant and eluting (MRE) haemagglutinins, fimbriae and surface structure in strains of *Haemophilus*. FEMS Microbiol. Lett. **10**:235–240.
35. **Short, J. A., C. M. Thorley, and P. D. Walker.** 1976. An electron microscope study of *Bacteroides nodusus*: ultrastructure of organisms from primary isolates and different colony types. J. Appl. Bacteriol. **40**:311–315.
36. **Silverman, M., and M. Simon.** 1980. Phase variation: genetic analysis of switching mutants. Cell **19**:845–854.
37. **Smit, G., J. W. Kijne, and B. J. J. Lugtenberg.** 1986. Correlation between extracellular fibrils and attachment of *Rhizobium leguminosarum* to pea root hair tips. J. Bacteriol. **168**:821–827.
38. **Stemmer, W. P. C., and L. Sequeira.** 1985. Possible role of fimbriae in attachment of plant-associated bacteria to plant cell walls, p. 199–201. *In* A. A. Szalay and R. P. Legocky (ed.), Proceedings of the 2nd International Symposium on the Molecular Genetics of the Bacteria-Plant Interaction. Media Services. Cornell University, Ithaca, N.Y.
39. **Steven, A. C., M. E. Bisher, B. L. Trus, D. Thomas, J. M. Zhang, and J. L. Cowell.** 1986. Helical structure of *Bordetella pertussis* fimbriae. J. Bacteriol. **167**:968–974.
40. **Takenaka, A., K. Oishi, and K. Aida.** 1979. Sugar specificity of the hemagglutinins produced by soil bacteria. J. Gen. Appl. Microbiol. **25**:397–400.
41. **Thorley, C. M.** 1976. A simplified method for the isolation of *Bacteroides nodosus* from bovine foot-rot and studies on its colonial morphology and serology. J. Appl. Bacteriol. **40**:301–304.
42. **Vesper, S. J., and W. D. Bauer.** 1985. Characterization of *Rhizobium* attachment to soybean roots. Symbiosis **1**:139–162.
43. **Vesper, S. J., and W. D. Bauer.** 1986. Role of pili (fimbriae) in attachment of *Bradyrhizobium japonicum* to soybean roots. Appl. Environ. Microbiol. **52**:134–141.
44. **Wadstrom, T., C. J. Smyth, A. Faris, P. Jonsson, and J. H. Freer.** 1978. Hydrophobic adsorptive and hemagglutinating properties of enterotoxigenic *Escherichia coli* with different colonizing factors: K88, K99, and colonization factor antigens and adherence factor, p. 29–45. *In* S. Acres (ed.), Second International Symposium on Neonatal Diarrhea. Veterinary Infectious Disease Organization, Saskatoon, Saskatchewan, Canada.
45. **Watts, T. H., C. M. Kay, and W. Paranchych.** 1983. Spectral properties of three quaternary arrangements of *Pseudomonas pilin*. Biochemistry **22**:3640–3646.
46. **Weller, D. M., and R. J. Cook.** 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology **73**:463–469.
47. **Woods, D. E., D. C. Strauss, G. J. Waldermar, Jr., V. K. Berry, and J. A. Bass.** 1980. Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. Infect. Immun. **29**:1146–1151.
48. **Wray, W., T. Boulikas, V. P. Wray, and R. Hancock.** 1981. Silver staining of proteins in polycrylamide gels. Anal. Biochem. **118**:197–203.
49. **Young, D. H., W. P. C. Stemmer, and L. Sequeira.** 1985. Reassembly of a fimbrial hemagglutinin from *Pseudomonas solanaceraum* after purification of the subunit by preparative sodium dodecyl sulfate-polycrylamide gel electrophoresis. Appl. Environ. Microbiol. **50**:605–610.