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Isolation and Characterization of Mutants with Lesions Affecting Pellicle Formation and Erythrocyte Agglutination by Type 1 Piliated *Escherichia coli*

SANDRA L. HARRIS, DAVID A. ELLIOTT, MICHAEL C. BLAKE, LISA M. MUST, MARK MESSENGER, AND PAUL E. ORNDORFF*

Department of Microbiology, Pathology and Parasitology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606

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The product of the *pilE* (also called *fimH*) gene is a minor component of type 1 pili in *Escherichia coli*. Mutants that have insertions in the *pilE* gene are fully piliated but unable to bind to and agglutinate guinea pig erythrocytes, a characteristic of wild-type type 1 piliated *E. coli*. In this paper we describe the isolation of 48 mutants with point lesions that map to the *pilE* gene. Such mutants were isolated by using *mutT* mutagenesis and an enrichment procedure devised to favor the growth of individuals that could form a pellicle in static broth containing α-methylmannoside, an inhibitor of erythrocyte binding and pellicle formation. Results indicated that the enrichment favored mutants expressing *pilE* gene products that were defective in mediating erythrocyte binding. Characterization of 12 of the mutants in greater detail revealed that certain lesions affected pilus number and length. In addition, a mutant that was temperature sensitive for erythrocyte binding was isolated and used to provide evidence that pellicle formation relies on the intercellular interaction of *pilE* gene products. Our results suggest a molecular explanation for the old and paradoxical observations connecting pellicle formation and erythrocyte agglutination by type 1 piliated *E. coli*.

Type 1 pili (fimbriae) are filamentous proteinaceous appendages expressed by several members of the family *Enterobacteriaceae* (11). Type 1 pili mediate bacterial binding to a variety of eucaryotic cells through a mannose-sensitive interaction with a receptor on the eucaryotic cell surface (8, 12, 34). *Escherichia coli* type 1 pili are about 5 nm wide and 1 to 2 μm long. They are composed principally of a single protein subunit, pilin, arrayed in a helical fashion around a hollow core (13).

Until recently, it was assumed that type 1 pili were composed exclusively of pilin monomers. However, work from a number of laboratories (1, 2, 13–15, 18, 21, 22, 24) has suggested that in addition to the pilin subunit there are at least three other minor components isolated with pili and assumed to be polymerized along with the pilin monomers during pilus biosynthesis (14, 18). Exactly how these minor components are arranged in the pilus shaft has been the subject of some attention and speculation (2, 13, 20, 24, 25).

Our laboratory has been particularly interested in one of the minor pilus components, the product of a gene we termed *pilE* (21, 22), which has also been called *fimH* (1, 2, 15, 18). The 31-kDa *pilE* product is necessary for type 1 piliated cells to bind to and agglutinate guinea pig erythrocytes (21, 22). Biochemical evidence has suggested that the *pilE* gene product is a minor pilus component that is located at the tip of the pilus and that it may also be intercalated in the shaft (2, 13). Recent evidence indicates that the *pilE* gene product acts directly as the agglutinin (17). However, the binding activity of the *pilE* product may be influenced by adjacent pilus components, since mutations that abolish pilus assembly also abolish (21) or at least reduce (16) receptor binding. We thus cannot formally eliminate the role of the other pilus components in influencing binding.

* Corresponding author.

It was initially thought that the *pilE* gene product was required solely for erythrocyte binding (22). However, subsequent work in our laboratory has revealed that, in addition to binding, pilus length is also influenced in some *pilE* mutants. Insertion mutations that fall in the 5′ half of the *pilE* gene produce mutants that have long pili and fail to bind erythrocytes, whereas insertions toward the 3′ end produce mutants that have normal-length pili but still fail to agglutinate erythrocytes. The insertions affecting length and binding had initially been attributed to the loss of a separate gene (*pilF*) whose product now appears to have resulted either from premature transcription termination of the *pilE* gene or breakdown of the *pilE* product. The association of a second phenotype (long piliation) with certain alleles of the *pilE* gene product suggests that this minor component influences pilus morphology as well as pilus binding.

During the isolation of insertion mutations in *pilE*, we also devised an enrichment procedure for isolating mutants with point lesions in *pilE*. The enrichment procedure was based upon early and puzzling observations that both pellicle formation and erythrocyte agglutination, carried out by type 1 piliated *E. coli*, are inhibited by the same compounds (11, 27, 28). Herein we describe the methods devised for the mutagenesis, enrichment, and isolation of 48 *pilE* mutants. Also, we describe selected properties of 12 of the mutants.

MATERIALS AND METHODS

Bacterial and bacteriophage strains, plasmids, and media. The bacterial strains (all *E. coli* K-12 derivatives), bacteriophage strains, and plasmids used or isolated in this investigation are listed in Table 1. Media consisted of L agar and L broth (23). Antibiotics were added as previously described (31). Minimal medium was minA medium supplemented with amino acids as described by Miller (23).

Genetic techniques. Generalized transduction with P1 *vir* was carried out as described by Miller (23).

TABLE 1. Bacteria, bacteriophages, and plasmids used in this study

Bacterium, bacteriophage, or plasmid	Description	Source or reference
E. coli		
SS320	*azi-9 pro-48 lacZ118*(Oc)[a] *lacI22 trpA9605*(Am) *his-85*(Am) *gyrA19 rpsL171 metE70 trpR55* λ⁻	Barbara Bachmann
SY805	*trpA lacZ mutT1*	R. Isberg
SuIII	(ϕ80) (ϕ80d SuIII[*supF*])	P. Bassford
ORN109	*thr leu proA2 lacY1 galK his argE rpsL supE mtl xyl recBC sbcB* (has Tn*10* between *hsd* and *serB*), Tet[r] Pil⁺	33
ORN115	*thr-1 leuB thi-1* Δ(*argF-lac*)*U169 xyl-7 ara-13 mtl-2 gal-6 rpsL tonA2 supE44 pilG*	33
ORN134	ORN115, except *pilE'-kan recA13*	22
ORN140	ORN115, except *pilE104*::Tn*5*	22
ORN147	ORN115, except *pilA'-kan*	22
ORN177	ORN147, except Mal⁺	P1 transduction from ORN109
ORN155	ORN115, except with Tn*5* insertion 113 adjacent to *pilE*	This study
ORN184	ORN155, except Mal⁺	P1 transduction from ORN109
ORN156	ORN155, except *pilE229*	This study
ORN157	ORN155, except *pilE205*(Ts)	This study
ORN183	ORN155, except Mal⁺	P1 transduction from ORN109
ORN158[b]	ORN155, except *pilE208*	This study
ORN159	ORN155, except *pilE222*	This study
ORN160	ORN155, except *pilE241*	This study
ORN161	ORN155, except *pilE247*	This study
ORN162	ORN155, except *pilE204*	This study
ORN163	ORN155, except *pilE218*	This study
ORN164[c]	ORN155, except *pilE236*	This study
ORN165	ORN155, except *pilE244*	This study
ORN166[b]	ORN155, except *pilE242*	This study
ORN167	ORN155, except *pilE246*	This study
ORN168	SS320, except *pilG zcg*::Tn*10* Trp⁺	P1 transduction from ORN126 (36)
ORN169	ORN168, except *pilE'-kan*	P1 transduction from ORN134
ORN170	ORN169, except (ϕ80d *supF*)	This study
Bacteriophages		
P1	*vir*	Laboratory collection
λ	*h80* c⁻ Δ*int*	R. Isberg
ϕ80	Wild type	P. Bassford
Plasmids		
pBR322	ColE1, Ap[r] Tc[r]	6
pACYC184	P15A, Cm[r] Tc[r]	9
pORN127	pBR322 replicon *pilE*, Ap[r]	22
pORN134	pORN127 with *pilE105*::Tn*5*	22
pORN142	pORN127 (pBR322 replicon) with Tn*5* insertion 113 adjacent to *pilE*, Ap[r]	22
pORN145	pACYC184 replicon containing *pilE*	This study
pORN201 through pORN248	pORN142 containing point mutations conferring the PilE⁻ phenotype[d]	This study
pGF1B cua:Phe	Encodes tRNA suppressor gene specifying CUA for phenylalanine	26
pGF1B cua:Cys	Encodes tRNA supressor gene specifying CUA for cysteine	26
pORN143[e]	pACYC184 replicon containing tRNA supressor gene specifying CUA for phenylalanine, Cm[r]	This study
pORN144[e]	pACYC184 replicon containing tRNA supressor gene specifying CUA for cysteine, Cm[r]	This study

[a] Oc, Ochre mutation.
[b] A class III mutant; see the text and Fig. 3.
[c] A class II mutant; see the text and Fig. 3.
[d] The PilE⁻ phenotype is full piliation and failure to agglutinate guinea pig erythrocytes. This phenotype was produced when the plasmids were resident in strains ORN134, ORN140, and ORN156 through 167.
[e] pORN143 and pORN144 were constructed by excising the supressor tRNA genes from pGF1Bcua:Phe and pGF1Bcua:Cys with *Pvu*II, adding *Xho*I linkers, and religating with *Sal*I-cut pACYC184.

Plasmid DNA was introduced into cells by transformation (19) or by linear transformation as previously described (33).

Construction of the ϕ80d SuIII(*supF*) lysogen was accomplished by infecting strain ORN168 with a UV-induced lysate of strain SuIII (35). Trp⁺ His⁺ lysogens were selected on minimal medium after infection with various dilutions of the lysate. Lysogens formed by the lowest dilution of lysate were examined for the defective prophage and absence of the helper phage by using standard techniques (23).

Mutagenesis of pORN142 (Table 1, Fig. 1) by a *mutT* strain of *E. coli* (37) was accomplished by introducing the plasmid into strain SY805 by transformation. A pool of

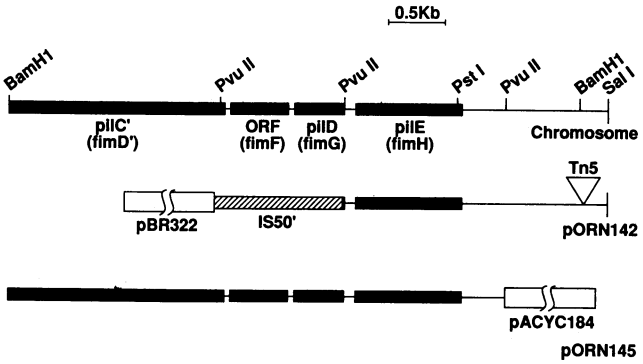

FIG. 1. *pilE* gene and surrounding region. The top diagram shows the physical map of the *pil* region. The placement of the *pilE* gene (21, 22), determined by insertion mutagenesis, is now brought into line with the sequence data published by Klemm and Christiansen (15) and our own unpublished sequence data. The genetic mnemonics in parenthesis are those of Klemm and Christiansen (15). Also, the *pilF* gene (22) is deleted, since it appears now that insertions defining that gene were, in fact, in the 5′ half of the *pilE* gene (see the text). Transcription of *pilE*, *pilD*, and the open reading frame (ORF) goes from right to left. The plasmid pORN142 was used for mutagenesis with *mutT*. The lower plasmid, pORN145, complemented all *pilE* point mutants isolated on pORN142.

approximately 1,000 transformant colonies was grown in L broth overnight, and the plasmid DNA was extracted and purified by cesium chloride density centrifugation.

Isolation of *pilE* point mutants. Mutant alleles of *pilE* were detected in the following way: *mutT*-mutagenized, purified plasmid DNA was introduced into strain ORN134 (*pilE'-kan recA13*) by transformation. Transformants were inoculated directly into 1.5 ml of L broth containing 25 mM α-methylmannoside and ampicillin, and the culture was grown without shaking. Typically, ca. 8,000 individual transformants were introduced into each culture. Twenty cultures were made per experiment, and each culture was passaged in the following way; after 48 h of growth, a sterile swab was touched to the surface of a culture and used to inoculate another tube containing the same medium. After three passages, a loopful of the culture was streaked onto an L-agar plate containing ampicillin, and 20 individual colonies were scored for the PilE$^-$ phenotype (full piliation but failure to agglutinate guinea pig erythrocytes). Only one PilE$^-$ colony was kept per tube. On average, 5 of the 20 tubes had at least one PilE$^-$ mutant. The range, in nine experiments, was 1 to 9 tubes of the 20. The lesions causing the PilE$^-$ phenotype were confirmed as mapping to the plasmid by extracting the plasmid DNA and reproducing the mutant phenotype in ORN134 transformants.

Introduction of *pilE* alleles into the chromosome. Mutant alleles of *pilE*, originally isolated on the pORN142 plasmid, were introduced into the chromosome of a *recBC sbcB* mutant of *E. coli* as described previously (33). Briefly, *Eco*RI-cut plasmid DNA was introduced into strain ORN109 by linear transformation, and Kan^r transformants were selected. A pool of the transformants was made by swabbing plates, usually containing approximately 300 colonies, and a transducing lysate was made from the pool. This lysate was used to infect ORN115. Kan^r Tet^r transductants of ORN115 bearing the linked *pilE* allele were identified by screening for the PilE$^-$ phenotype. From each isolate, another P1 lysate was made, and the linkage of the Tn*5* insertion to the mutant *pilE* allele was determined. In all cases presented herein, the linkage of Tn*5* to *pilE* was greater than 95% and the linkage to Tn*10* (between *hsd* and *serB* [4]) was ca. 8%, as would be predicted from the position of Tn*5* insertion 113 (22) (Table 1, Fig. 1). The final strains used in this study (ORN155 through ORN167) have Tn*5* insertion 113 adjacent to a particular *pilE* allele and do not have Tn*10*.

Detection of piliation. Piliation was detected by using guinea pig erythrocytes and antiserum raised against purified pili as previously described (30). For the assay of mutants bearing the *mutT*-induced mutations, comparisons were made with overnight static broth cultures of mutant and parental strains. Equal volumes of culture were compared for their ability to agglutinate guinea pig erythrocytes. These conditions ensured that similar numbers of cells were tested and that the physiological conditions of parental and mutant cultures were the same. Mutants with the PilE$^-$ phenotype agglutinate in antipili antiserum but do not agglutinate guinea pig erythrocytes (21, 22).

Recombinant DNA techniques. Conditions for restriction endonuclease digestion, agarose gel electrophoresis, and isolation and ligation of DNA fragments were as described previously (30–32).

Electron microscopy. Growth and treatment of cells for electron microscopy were as previously described (21, 22). Cells were examined with a Philips 410 transmission electron microscope after negative staining with 1% phosphotungstic acid. When mutants were divided into classes based upon aberrant pilus morphology, electron microscopic data was gathered blind; wild-type and mutant cultures were coded so that the person photographing representative cells did not know the identity of the strains. Also, preparations of cells and electron microscopy were performed on two separate occasions. Classes were established by a consensus of groupings made by two individuals, each ignorant of the grouping of the other.

RESULTS

Enrichment of *pilE* mutants in static broth containing α-methylmannoside. We developed the enrichment procedure described in Materials and Methods to favor the outgrowth of mutants that could form a pellicle (surface film) (7, 10, 28, 29) in medium containing α-methylmannoside, an analog of mannose that normally inhibits pellicle formation and erythrocyte binding (27–29). Mutants at the oxygen-rich

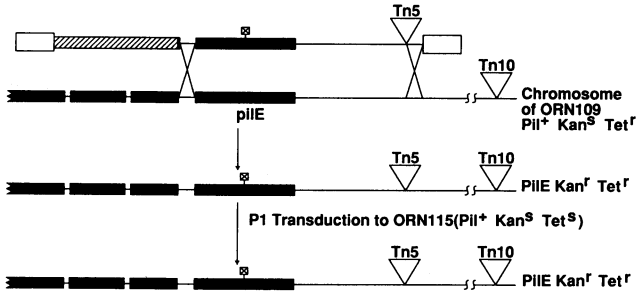

FIG. 2. Diagrammatic representation of the introduction of *pilE* point lesions into the chromosome of ORN115. Starting at the top, the small box with the "×" inside represents a lesion on one of the mutant *Eco*RI-cut pORN142 plasmids. The next line shows a Kan^r ORN109 transformant containing the *pilE* mutant allele. A P1 transducing lysate was made on a pool of the Kan^r transformants, and the lysate was used to infect ORN115, with selection for Kan^r Tet^r transductants. These transductants were screened for the PilE$^-$ phenotype. Subsequent constructions and genetic mapping were done by using P1 transduction.

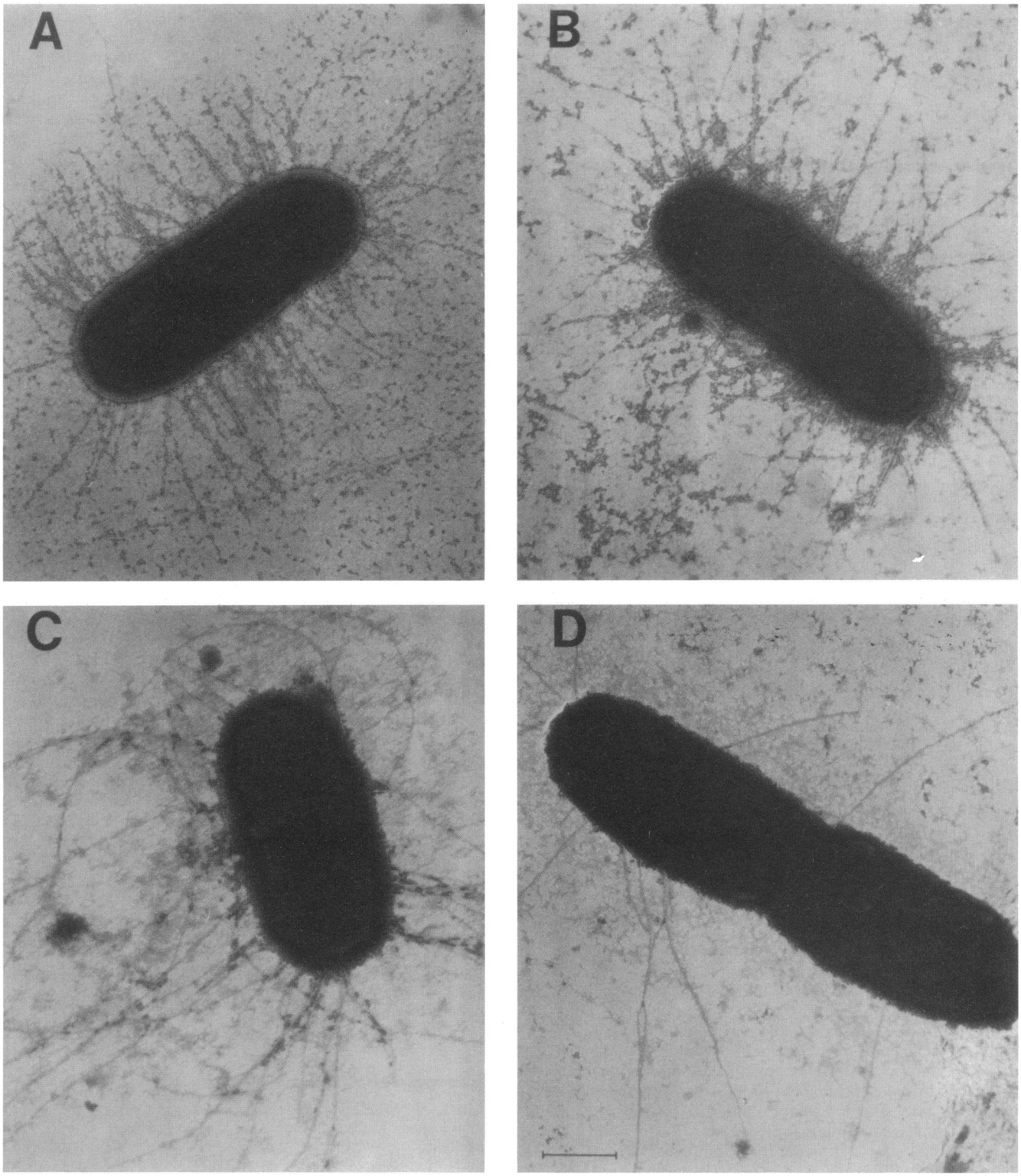

FIG. 3. Electron micrographs showing representative members of the three classes of *pilE* mutants. The classes were based upon aberrant pilus morphology or pilus number after introduction of 12 *pilE* mutant alleles into the chromosome of strain ORN115. (A) Control with the parental *pilE* allele (ORN155). (B) Class I mutant (ORN159). (C) Class II mutant (ORN164). (D) Class III mutant (ORN166). Bar, 0.5 μm.

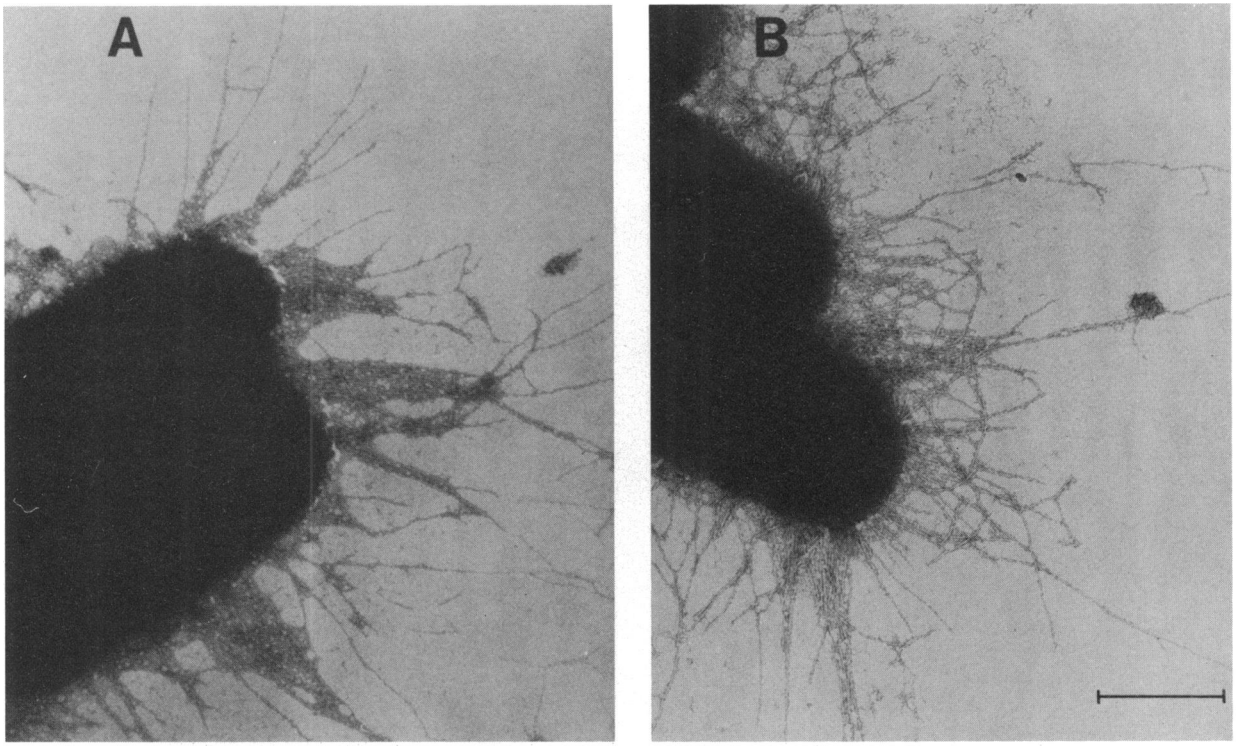

FIG. 4. Electron micrographs showing the piliation of strain ORN157 [*pilE205*(Ts)] when grown at 31°C (A) and 42°C (B). Bar, 0.5 μm.

surface of static broth outgrow individuals that cannot participate in pellicle formation. Early results with insertion mutations established that the *pilE* gene product was needed for normal enrichment to take place (i.e., in the absence of α-methylmannoside) and that at least one class of mutants favored by the α-methylmannoside enrichment procedure had the PilE$^-$ phenotype (full piliation but failure to agglutinate guinea pig erythrocytes).

In the current protocol, a subclone of the *pil* region (carried on plasmid pORN142) containing the *pilE* gene and an adjacent Tn*5* insertion was used as a target for *mutT* mutagenesis (Fig. 1). The mutagenesis was designed to favor missense mutations by taking advantage of the AT→CG transversions produced in *mutT* strains (3, 5, 37). The parental *pilE* gene on the pORN142 plasmid normally complements a *pilE'-kan* insertion mutation in the chromosome of ORN134 to produce a hemagglutinating (PilE$^+$) strain. However, after mutagenesis, introduction into the ORN134 strain, and α-methylmannoside enrichment, a high proportion of the isolates examined had the PilE$^-$ phenotype. A total of 48 *pilE* mutants were isolated by this procedure. In all 48 cases the PilE$^-$ phenotype was due to a lesion on the mutagenized plasmid.

Characterization of the *pilE* mutants. All of the 48 plasmids showed no signs of deletions or insertions and failed to restore hemagglutinating ability upon strain ORN134 containing the *pilE'-kan* insertion. The plasmids also failed to complement strain ORN140 carrying the *pilE104*::Tn*5* lesion (formerly called *pilF104*::Tn*5*) (22). The *pilE104*::Tn*5* allele has the Tn*5* insertion much nearer the 5′ end of the *pilE* gene than the *pilE'-kan* insertion (22). These results suggested that the mutant phenotype was conferred by a small lesion and did not result from an interaction of the plasmid-encoded mutant product with a possible partial product from the chromosomal allele.

Mapping of the lesions by complementation with pORN145 (Fig. 1) revealed that the lesions map in or very close to *pilE*.

None of the 48 lesions appeared to be nonsense mutations, since none of the three nonsense supressors tested (*supF* in strain ORN170 or the supressors carried by pORN143 and pORN144; Table 1) were effective in restoring the parental phenotype.

Characterization of the chromosomal *pilE* mutants. Twelve of the 48 lesions were introduced into the chromosome of ORN115 as described in Materials and Methods (Fig. 2). Since it was necessary to score for the mutant phenotype to conclude that the mutant allele had been introduced, we took the precaution of examining the possibility that we were introducing spurious mutations into *pilE*. This was done by examining Kan^r recombinants resulting from the introduction of the *pilE205* (Ts) allele carried by pORN205 into the chromosome of ORN109. Earlier screening had revealed that pORN205 conferred a temperature-sensitive *pilE* phenotype upon ORN134; that is, ORN134(pORN134) was PilE$^+$ at 31°C and PilE$^-$ at 42°C. Of the 50 PilE$^-$ recombinants resulting from the linear transformation of ORN109, all were found to be temperature sensitive. This suggested that the procedure for introducing the lesion was faithfully introducing the plasmid-encoded mutation into the chromosome.

We examined the possibility that one (or more) of the chromosomal mutant alleles could be complemented by one (or more) of the 48 plasmid-encoded alleles. This was done by introducing each of the 48 plasmids into each of the 12 strains containing the chromosomal mutant *pilE* alleles and scoring for erythrocyte agglutination. There was no indication of complementation in any of the combinations.

Alleles of *pilE* causing aberrant pilus morphology. Each of the 12 chromosomal mutants (strains ORN156 through ORN167) and the wild-type strain ORN155 were examined

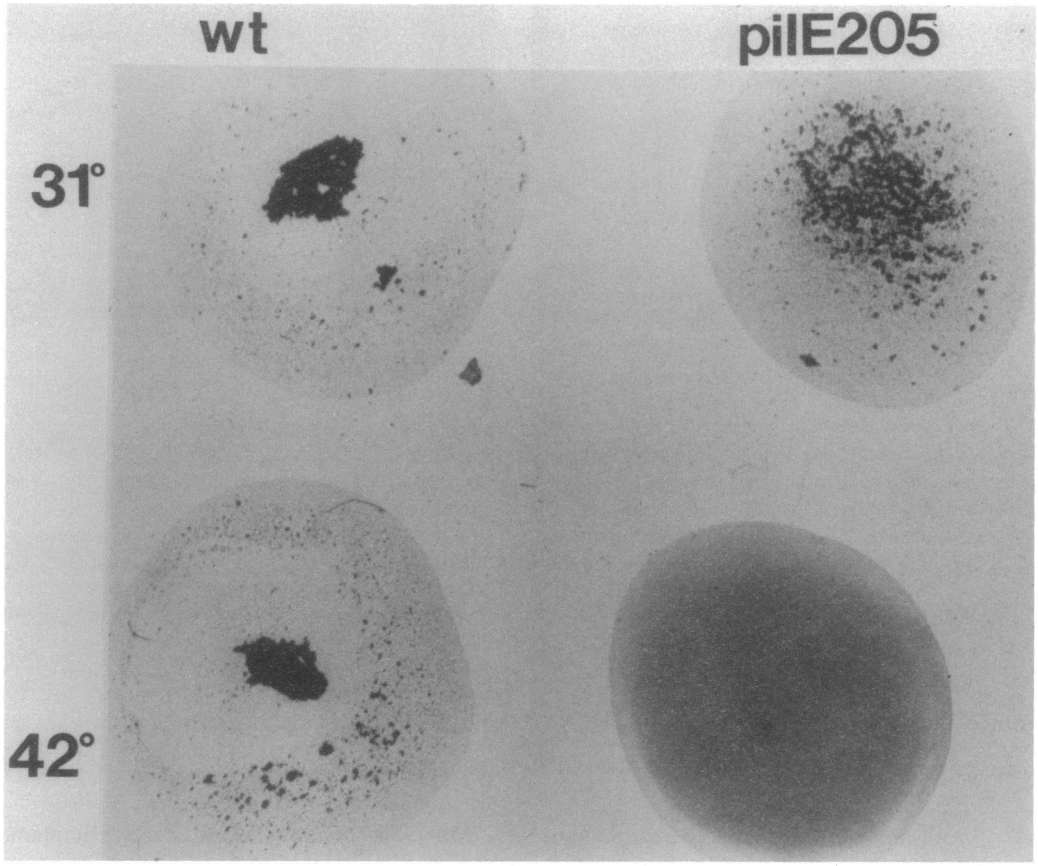

FIG. 5. Temperature-sensitive nature the *pilE205* allele is demonstrated by the inability of the temperature-sensitive mutant to agglutinate guinea pig erythrocytes after being grown at 42°C. Agglutination of erythrocytes by ORN155 with the wild-type (wt) *pilE* allele and grown at 31 or 42°C is shown on the left. The agglutinating ability of strain ORN157 [bearing the pilE205(Ts) allele] and grown under conditions identical to those for the wild type is shown on the right.

by electron microscopy. Three phenotypic classes could be distinguished (Fig. 3). Class I mutants had normal levels of piliation and normal-length pili. Class II mutants had longer pili but normal numbers. Class III mutants had dramatically fewer pili, but the pili that were present were very long. Of the 12 mutants, 9 were class I, 1 was class II, and 2 were class III mutants. The class II and III mutants are designated as such in Table 1. All other chromosomal mutants listed in Table 1 are class I mutants.

Temperature-sensitive *pilE* mutation. As mentioned above, one of the 48 mutants exhibited a conditional temperature-sensitive phenotype. Strain ORN157, bearing the *pilE205* (Ts) allele, was normally piliated at both 31 and 42°C (Fig. 4), but growth temperature had a dramatic effect upon the ability of the temperature-sensitive mutant to agglutinate guinea pig erythrocytes (Fig. 5). The temperature-sensitive phenotype was somewhat leaky; if the temperature-sensitive mutant, grown at 41°C, was incubated with erythrocytes for an extended period, there was some agglutination (strains completely lacking *pilE* do not show a trace of hemagglutination).

Role of *pilE* in pellicle formation. Since pellicles were formed in the presence of α-methylmannoside in the *pilE* point mutants but were not detectable at all in the complete absence of the *pilE* product, we devised a test to see what component parts of the mutants were interacting to form a pellicle. The mutant carrying the *pilE205*(Ts) allele formed a

TABLE 2. Filtration assay[a]

Strains mixed	Piliation genotype		Ratio after filtration[b]	SD
	Mal⁻	Mal⁺		
Set I				
ORN157-ORN183	*pilE*(Ts)	*pilE*(Ts)	1.26	0.28
ORN157-ORN184	*pilE*(Ts)	Parental[c]	12.95	8.13
ORN155-ORN183	Parental	*pilE*(Ts)	0.17	0.07
ORN155-ORN184	Parental	Parental	0.82	0.19
Set II				
ORN157-ORN183	*pilE*(Ts)	*pilE*(Ts)	1.14	0.64
ORN157-ORN177	*pilE*(Ts)	*pilA'-kan*[d]	10.24	6.05
ORN147-ORN183	*pilA'-kan*	*pilE*(Ts)	0.02	0.01
ORN147-ORN177	*pilA'-kan*	*pilA'-kan*	1.23	0.26

[a] Overnight cultures grown at 31°C in L broth containing 25 mM α-methyl mannoside were diluted 1:100 in prewarmed fresh medium, mixed 1:1 (5 ml each), and incubated for 6 h at 42°C with very slow shaking (25 rpm) in 125-ml Erlenmeyer flasks. This procedure allowed contact of all cells but minimally disturbed the aggregates (pellicle fragments) that formed. After the incubation, a wide-bore, 25-ml pipette was used to remove 5 ml of the cultures. The samples were placed in a sterile filter apparatus containing a filter of 5 μm pore size and allowed to drip through. A sample from the upper chamber was taken immediately after addition, diluted, and plated on maltose-tetrazolium agar plates (35). Bacterial counts from this unfiltered sample were used to normalize results obtained by washing the filters and plating the contents.

[b] Ratios of prefiltered samples were used to normalize results to 1. Thus, deviation from unity represents an effect of the filtration.

[c] The parental phenotype means normal piliation with a full complement of the genes required for piliation.

[d] The lesion in the *pilA* gene eliminates the pilus subunit, precluding piliation.

strong pellicle at 42°C but virtually no pellicle at 31°C in the presence of α-methylmannoside. We grew mutant, parental, and nonpiliated strains at 31°C, diluted them, mixed them pairwise in approximately equal proportions (each member of the pair was genetically marked by its ability to utilize maltose), and then let them grow together at 42°C. Cells participating in pellicle formation under these conditions were significantly impeded from passing through a 5-μm-pore-size filter. By measuring the ratio of cells in the upper chamber of the filter apparatus before filtering and comparing it with the ratio on the filter after filtering, we obtained a relative measure of the types of cells participating in pellicle formation. In all cases tested, the *pilE* mutant individuals were found to form pellicles to the exclusion of the parental (set I, Table 2) and nonpiliated strains (set II, Table 2). This result indicated that pellicle formation may be effected via the intercellular interaction of *pilE* gene products.

DISCUSSION

The *pilE* gene product is a minor component of type 1 pili and is necessary for the mannose-sensitive agglutination of guinea pig erythrocytes by type 1 piliated *E. coli*. Insertion mutations that map to *pilE* abolish agglutinating ability without affecting the ability of the mutants to polymerize pilin monomers into pili (21). In this paper we describe an enrichment procedure we devised to obtain 48 mutants with apparent point lesions, induced by *mutT* mutagenesis, that map to the *pilE* gene. All of these mutants could form pellicles in the presence of α-methylmannoside and failed to agglutinate erythrocytes. Further characterization of 12 of the mutants revealed an effect of some of the *pilE* lesions on pilus morphology. Experiments with one temperature-sensitive mutant suggested that intercellular interactions between *pilE* gene products facilitate pellicle formation.

It has been known for over 30 years that type 1 piliated *E. coli* form a pellicle (surface film) on the surface of static broth cultures (10). Past literature attributes pellicle formation to the hydrophobic character of pili (reviewed in reference 11), and this characteristic would indeed seem to be a prerequisite for piliated cells to be at an air-water interface—although this might not explain the formation of the film. Oddly, pellicle formation is inhibited by the same compounds that inhibit the agglutination of erythrocytes by type 1 pili (11, 27, 28). The likely connection between these two properties (pellicle formation and hemagglutination) suggested to us the enrichment procedure described herein. The enrichment resulted in the isolation of 48 *pilE* mutants that were indeed aberrant in erythrocyte binding. A major strength of the enrichment was that it demanded that the mutants still produce a phenotype (pellicle formation) associated with the *pilE* product. Consequently, we believe that all of the point mutants produce a *pilE* gene product, but one defective in erythrocyte binding.

In addition to conferring pellicle formation in the presence of α-methylmannoside and defective erythrocyte binding, some of the *pilE* lesions produced mutants that exhibited anomalous pilus number and morphology. However, since most of the mutants produced normal-looking pili, we think it likely that only extremely aberrant *pilE* products affect pilus morphology. Nevertheless, the study of such mutants may provide a clue to the manner of assembly of these supramolecular structures.

Apart from the mutants that conferred a change in pilus number and morphology, a temperature-sensitive mutant was identified. We employed this mutant as a tool to address the question of what cell surface component(s) the mutant pilus product bound in effecting pellicle formation. The results supported the idea that *pilE* gene products in pili on adjacent cells interact to form a pellicle, although this was not shown directly.

In conclusion, our mutant isolation indicated that both pellicle formation and erythrocyte binding are related through their dependence upon the *pilE* gene product. We suspect that the reason that α-methylmannoside normally inhibits guinea pig erythrocyte agglutination as well as pellicle formation is because it blocks a region of the *pilE* molecule that is required for both processes. Consequently, if one isolates a mutant that is "blind" to the inhibitory effect of α-methylmannoside on pellicle formation, this same mutant is likely to be defective for erythrocyte binding. We hope that these mutants will prove useful in dissecting receptor-ligand interactions by defining regions of the *pilE* protein that are important in erythrocyte binding. In addition, we hope that some of the lesions we have isolated will prove useful in examining the steps in the biogenesis of pili. Clearly, some of the lesions have a dramatic effect on pilus number and morphology.

ACKNOWLEDGMENT

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