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# Identification and Characterization of Genes Determining Receptor Binding and Pilus Length of *Escherichia coli* Type 1 Pili

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**We describe the identification and characterization of two genes and their gene products responsible for determining receptor binding and pilus length in type 1-piliated *Escherichia coli*. One gene, *pilE*, conferred the ability of piliated cells to agglutinate guinea pig erythrocytes. The other gene, *pilF*, determined pilus length, in that mutants having lesions in *pilF* had very long pili. The two genes were detected after Tn*5* mutagenesis of a cloned segment of DNA that normally complemented a *pilE* lesion in the chromosome. Thus, lesions in *pilE* or *pilF* on the cloned segment resulted in mutants having the PilE$^-$ phenotype (piliated but unable to agglutinate erythrocytes). Introduction of the plasmid-encoded mutant alleles of *pilE* and *pilF* into the chromosome followed by electron microscopic examination of the mutants showed that only lesions in *pilF* conferred the striking increase in pilus length. Mutations in *pilF* could be complemented in *trans* by the original cloned segment to produce cells with parental-length pili. Minicell transcription and translation of the cloned *pilE* and *pilF* genes having representative Tn*5* insertion mutations showed that the *pilE* gene product was a protein of ca. 31 kilodaltons and that the *pilF* gene product was a protein of ca. 18 kilodaltons. We believe that the *pilF* gene product may act as a competititve inhibitor of pilus polymerization. Thus, pilus length may be controlled by the ratio of pilin to *pilF* gene product present within the cell.**

Type 1 pili of *Escherichia coli* provide a convenient model for studying the control and assembly of supramolecular extracellular structures. Also, they provide a model for cell-cell interaction, since type 1 pili are required to mediate attachment of *E. coli* to a variety of eucaryotic cells through a mannose-sensitive interaction with a receptor on the eucaryotic cell (4).

One interesting facet of the assembly of type 1 pili concerns how the cell monitors the number of pili it has and how long those pili are. These two considerations would seem to be important for the cell to conserve energy. However, since pili are on the outside of the cell, it is unclear how the cell would keep track of these two parameters. It is apparent from recent work that at least one mechanism exists for controlling the number of pili per cell. This fact is indicated by the existence of hyperpiliated mutants (13). Since hyperpiliated mutants also have longer pili (13), one might infer that the cell has a way to monitor pilus length also. However, no such pilus length mutants have been isolated.

Another interesting feature of type 1 pili concerns their involvement in receptor-ligand interactions. Recently, it has become evident that while type 1 pili are required for receptor binding, their presence is not sufficient for binding (8). This fact is indicated by the existence of fully piliated mutants that fail to agglutinate guinea pig erythrocytes (8). The receptor-binding function requires, in addition to polymerized pili, the product of the *pilE* gene (8). The *pilE* gene product is distinct from pilin (the *pilA* gene product), which makes up the pilus fiber. The precise role of the *pilE* gene product in erythrocyte agglutination is not known. For the purpose of experimentation, it is assumed either that the *pilE* gene product acts as the true adhesive component (adhesin), requiring the pilus only for proper presentation, or that the *pilE* gene product acts indirectly, for example, conferring receptor-binding capacity on the pilin monomer (8, 10).

In the case of Pap pili (pili associated with some strains of *E. coli* causing pyelonephritis), the adhesin is distinct from the pilus fiber (6, 11, 19). The adhesin in this case appears to be a minor component of pili (7). If the Pap model holds true for type 1 pili, then the *pilE* gene product may be a minor component of pili. However, for the purpose of experimentation, it is assumed either that the *pilE* gene product acts as the true adhesin, requiring the pilus only for proper presentation, or that the *pilE* gene product acts indirectly, for example, conferring receptor-binding function on the pilin monomer (8, 10).

The *pilE* locus has been mapped and shown to act in *trans* (8). However, the gene product has not been observed. In this communication, we report the transcription and translation of the *pilE* gene and describe the protein product. In the course of isolating insertion mutations that conferred the PilE$^-$ phenotype (antigenically piliated but unable to agglutinate erythrocytes), we found a second, previously undescribed gene adjacent to *pilE* which we have termed *pilF*. Insertion mutations in *pilF* also produced the PilE$^-$ phenotype. However, examination of *pilF* mutants revealed that they all had very long pili. The presence of the *pilF* gene suggests that the cell does, in fact, have a way to control pilus length.

## MATERIALS AND METHODS

**Bacterial and bacteriophage strains, plasmids, and media.** The bacterial strains (all *E. coli* K-12 derivatives), the bacteriophage strains, and previously described plasmids used in this investigation are listed in Table 1. Plasmids that were constructed specifically for this work are described in Results. Media consisted of brain heart infusion broth (Difco Laboratories), L-agar and L-broth (9); antibiotics were added as previously described (12).

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

| Bacterium, bacteriophage, or plasmid | Description | Source or reference |
|---|---|---|
| **Bacteria** | | |
| MC1000 | *araD139* Δ(*araABOIC leu*)*7697* Δ(*lacIPOZY*)*X74 galU galK rpsL thi* | 3 |
| EC901 | *leu-6 argE3 proA2 lacY1 his-4 thi-1 galK2 ara-14 xyl-7 srl-31 hsdR4 recA13 srl*::Tn*10* | 17 |
| ORN103 | *thr-1 leu-6 thi-1* Δ(*argF-lac*)*U169 xyl-7 ara-13 mtl-2 gal-6 rpsL fhuA2 minA minB recA13* Δ(*pilABCDFE hyp*) | 14 |
| ORN115 | *thr-1 leuB thi-1* Δ(*argF-lac*)*U169 xyl-7 ara-13 mtl-2 gal-6 rpsL tonA2 supE44 pilG* (does not exhibit phase variation of piliation) | 14, 18 |
| ORN122 | *thr-1 leuB thi-1 lacY1 malA1 xyl-7 ara-13 mtl-2 gal-6 rpsL tonA2 supE44 pilA'-kan* Tet$^r$ | 8 |
| ORN123 | *thr leu proA2 lacY1 galK his argE rpsL supE mtl xyl recBC sbcB pilE'-kan* (has Tn*10* between *hsd* and *serB*) Tet$^r$ | 8 |
| ORN147 | ORN115 except *pilA'-kan* | P1 transduction from ORN122 |
| ORN133 | ORN115 except *pilE'-kan* Tet$^s$ | P1 transduction from ORN123 |
| ORN134 | ORN133 except *recA13* | P1 transduction from EC901 and ORN103 |
| ORN135 | ORN115 except *pilE105*::Tn*5* | This study |
| ORN136 | ORN115 except *pilE114*::Tn*5* | This study |
| ORN137 | ORN115 except *pilF101*::Tn*5* | This study |
| ORN138 | ORN115 except *pilF102*::Tn*5* | This study |
| ORN139 | ORN115 except *pilF103*::Tn*5* | This study |
| ORN140 | ORN115 except *pilF104*::Tn*5* | This study |
| ORN141 | ORN115 except *pilF106*::Tn*5* | This study |
| ORN142 | ORN115 except *pilF107*::Tn*5* | This study |
| ORN143 | ORN115 except *pilF108*::Tn*5* | This study |
| ORN144 | ORN115 except *pilF109*::Tn*5* | This study |
| ORN145 | ORN115 except *pilF117*::Tn*5* | This study |
| ORN146 | ORN115 except *pilF118*::Tn*5* | This study |
| **Bacteriophages** | | |
| λ Tn*5* | *c*I857 *b221 rex*::Tn*5 O*am *P*am80 | 16 |
| P1 | *vir* | Laboratory collection |
| **Plasmids** | | |
| pBR322 | ColE1 Ap$^r$ Tc$^r$ | 2 |
| pRN2010 | ColE1 Sp$^r$ Tc$^r$ | 12 |
| pORN127[a] | pBR322 *pilE pilF* Ap$^r$ | 8 |
| pORN104 | pRN2010 *pilABCDFE hyp* Sp$^r$ | 12 |
| pORN134 | pORN127 *pilE105*::Tn*5* Km$^r$ Ap$^r$ | This study |
| pORN135 | pORN127 *pilF104*::Tn*5* Km$^r$ Ap$^r$ | This study |
| pORN139 | pORN127 *pilF108*::Tn*5* Km$^r$ Ap$^r$ | This study |
| pORN136 | pORN127::Tn*5*(119) (*pilE pilF*) Km$^r$ Ap$^r$ | This study |

[a] This plasmid was initially thought to carry the gene adjacent to *pilF*, *pilD* (8). This was in error. At most, only a portion of the *pilD* gene is present in this subclone.

**Recombinant DNA techniques.** Conditions for restriction endonuclease mapping, agarose gel electrophoresis, isolation of restriction fragments, ligation, and transformation of plasmid DNA have been previously described (12, 14).

**Genetic techniques.** P1 transduction was done as described by Miller (9). Tn*5* insertion mutagenesis of pORN127 was accomplished as previously described (12). Reintroduction of mutant alleles of *pilE* and *pilF* into the chromosome of *recBC sbcB* mutants and subsequent transfer of the mutant alleles by P1 transduction have been described previously (14).

**Transcription and translation of plasmid DNA.** Plasmid DNA was transcribed and translated in minicells (1). Minicells were isolated from strain ORN103 after 15 h of growth in brain heart infusion broth by successive differential and sucrose density centrifugations. Minicells were labeled in the presence of ca. 50 μCi of $^{35}$S-cysteine. Radiolabeling procedures, polyacrylamide gel electrophoresis, and autoradiography were done as previously described (12).

**Assays for piliation.** Bacterial agglutination in the presence of antiserum raised against purified type 1 pili and hemagglutination of guinea pig erythrocytes were accomplished as previously described (12). The PilE$^-$ phenotype is defined by the failure of mutants to agglutinate guinea pig erythrocytes while maintaining the ability to agglutinate in antipilus antiserum (8).

**Electron microscopy.** Strains used for electron microscopic examination were grown overnight in L-broth at 37°C without shaking. A drop of the overnight culture was placed on a Formvar-coated grid, the grid was rinsed, and the cells were stained with 2% phosphotungstic acid. Negatively stained preparations were examined on a Philips 410 transmission electron microscope.

## RESULTS

**Isolation and mapping of Tn*5* insertion mutations in *pilE* and *pilF*.** To direct insertion mutations to the *pilE* region, we used a subclone of original parental plasmid pSH2 (12), pORN127 (*pilE pilF*) (Table 1 and Fig. 1), as a target for Tn*5* insertions. Random insertion mutations in pORN127 were

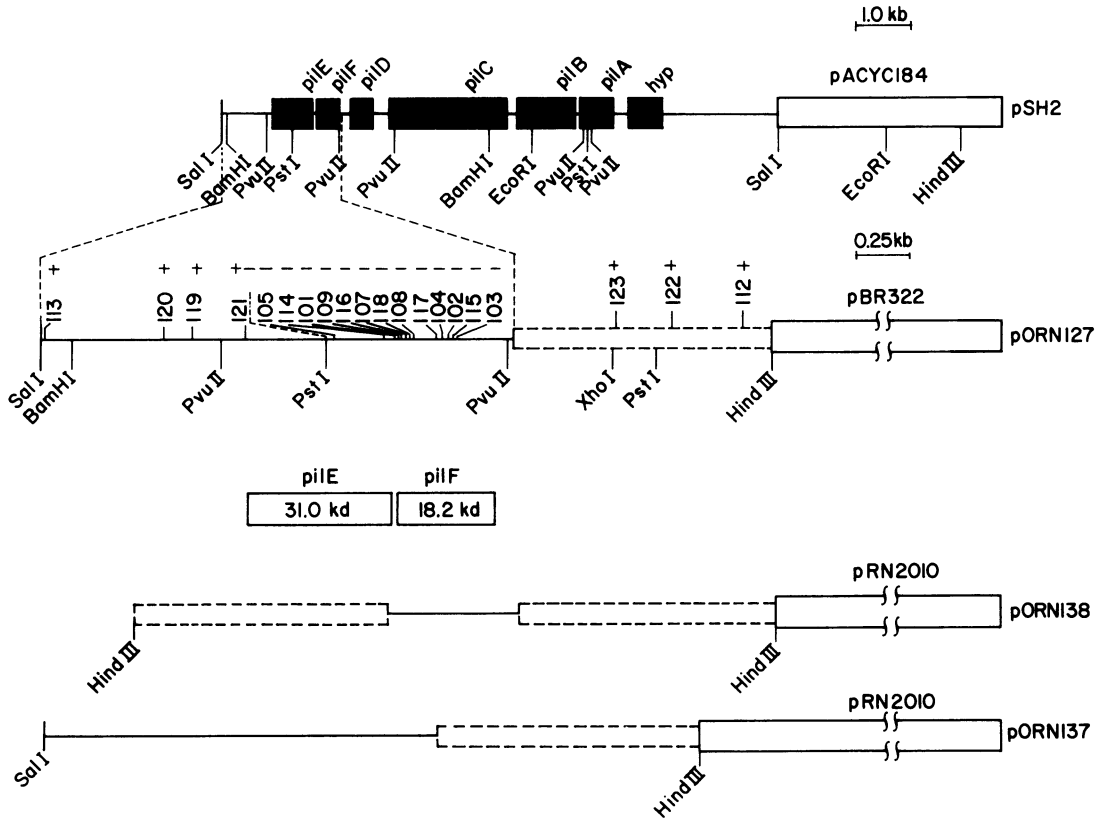


FIG. 1. Location of Tn*5* insertion mutations defining the *pilE* and *pilF* genes and subcloned regions containing *pilE* or *pilF*. Genes of the *pil* region are shown as black boxes. Each box is proportional to the size of the gene product. The subcloned region in pORN127 is shown with the locations of representative Tn*5* insertion mutations. Each insertion mutation has a number, and above each number is designated whether the insertion abolished *pilE* complementation (−) in strain ORN134 or had no effect on *pilE* complementation (+). A lesion at the *Pst*I site originally used to define the *pilE* gene is indicated by a dashed line. A portion of IS*50* (15) on plasmid pORN127 is designated by a dashed box. The *pilE* and *pilF* gene products represented by the open boxes show the molecular masses of the proteins (the 18.2-kDa [kd] *pilF* gene product is referred to in the text as an 18-kDa product). Below are shown two of the subcloned regions. pORN138 was subcloned from allele 114 and contains a portion of IS*50* on the left-hand end. pORN137 was subcloned from allele 117. The hatch marks indicate that the vectors are not drawn to scale. kb, kilobases.

isolated by extracting plasmid DNA from kanamycin-resistant (Kan$^r$) clones of MC1000(pORN127) following λ Tn*5* mutagenesis. Extracted plasmid DNA found to confer kanamycin resistance in strain ORN103 was used to transform strain ORN134 (*recA13 pilE'-kan*). Selection for ampicillin-resistant clones and screening of 200 of those clones for the PilE$^-$ phenotype produced 14 clones with insertion mutations in pORN127 that abrogated the normal complementation of the *pilE'-kan* lesion in the chromosome of ORN134. Insertion mutations conferring a PilE$^-$ phenotype and insertion mutations having no effect on complementation were mapped by restriction endonuclease digestion (12). Representative insertions and the phenotypes produced are shown in Fig. 1.

**Identification of the *pilE* and *pilF* gene products.** Transcription and translation of pORN127 and mutant derivatives in minicells revealed that insertion mutations that abolished *pilE* complementation also eliminated the production of either of two proteins (Fig. 2). One protein had a molecular mass of 31 kilodaltons (kDa); the other had a molecular mass of 18 kDa (Fig. 2A). Insertion mutations that eliminated the 31-kDa protein mapped to the left-hand end of the cluster of Tn*5* insertions abrogating *pilE* complementation (Fig. 1). Since this left-hand end was closest to the prototype lesion defining *pilE* (a deletion at the *Pst*I site [8]), the 31-kDa protein was designated the *pilE* gene product. The other protein whose absence was associated with the PilE$^-$ phenotype was an 18-kDa protein encoded by an adjacent gene which we termed *pilF* (Fig. 2A). Insertion mutations in *pilF* appeared to reduce the production of the *pilE* gene product in minicells (Fig. 2A), whereas insertion mutations in *pilE* had little effect on the production of the *pilF* gene product (Fig. 2B). We inferred from this result that the insertions in *pilF* were most likely polar on *pilE* expression. Subclones predicted to contain just an intact *pilF* gene (e.g., pORN138) expressed the *pilF* protein (Fig. 2C). However, we were unable to detect the synthesis of the *pilE* protein in similar subclones predicted to contain the *pilE* gene (e.g., pORN137; data not shown). The low production of the *pilF* and *pilE* gene products was consistently noted regardless of whether the minicells were labeled with [$^{35}$S]cysteine or [$^{35}$S]methionine.

**Discovery of the properties of *pilF*.** Tn*5* insertion mutations that eliminated the *pilF* protein produced the PilE$^-$ phenotype. However, we found that an additional property associated with *pilF* mutants was a rather dramatic increase in pilus length when *pilF*::Tn*5* alleles were introduced back into the chromosome by homologous recombination (14).

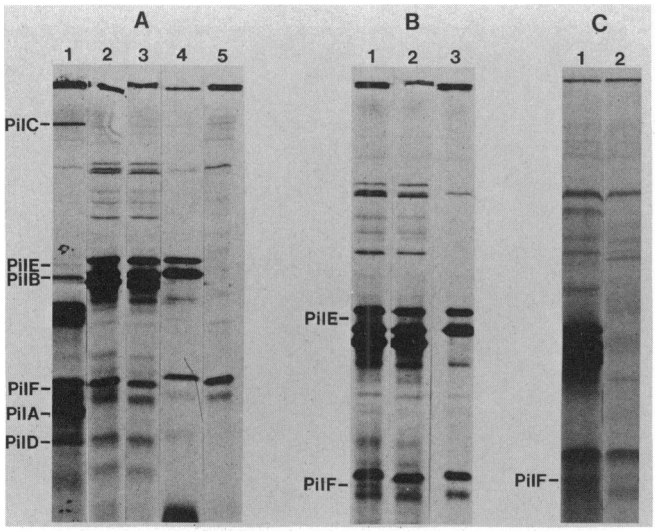


FIG. 2. (A) Autoradiogram of sodium dodecyl sulfate-polyacrylamide gel showing the $^{35}S$-labeled gene products of plasmids encoding *pilE* and *pilF* genes and showing the effects of an insertion mutation in *pilF* on the production of the *pilE* gene product. Lanes: 1, pORN104 (*hyp pilABCDFE*); 2, pORN136 (*pilE pilF*); 3, pORN139 (*pilE pilF108*::Tn*5*); 4, pBR322; 5, pRN2010. (B) Autoradiogram showing the effects of a lesion in *pilE* on the production of the *pilF* gene product. Lanes: 1, pORN136 (*pilE pilF*); 2, pORN134 (*pilE105*::Tn*5 pilF*); 3, pBR322. (C) Autoradiogram showing the gene products of a subclone containing the *pilF* gene. Lanes: 1, pORN136 (*pilE pilF*); 2, pORN138 (*pilF*). Approximate molecular masses are as follows: PilA, 17 kDa; PilB, 30 kDa; PilC, 86 kDA; PilD, 14 kDa; PilF, 18 kDa; and PilE, 31 kDa (the *hyp* gene product is not shown because it is weakly radiolabeled under these growth conditions).

Insertion mutations examined in this manner consisted of *pilF*::Tn*5* alleles 101, 102, 103, 104, 106, 107, 108, 109, 117, and 118. In contrast, when Tn*5* insertion mutations that eliminated the 31-kDa *pilE* gene product were introduced back into the chromosome, there was no increase in pilus length. Alleles of *pilE*::Tn*5* examined were *pilE105*::Tn*5* and *pilE114*::Tn*5*.

The average increase in pilus length in *pilF* mutants was ca. threefold, and a slight decrease in the number of pili per cell was consistently noticed (Fig. 3). Lesions in *pilF* could be complemented in *trans* by pORN127 (*pilE pilF*) (Fig. 3) and by pORN138 (which encodes just an intact *pilF* gene [Fig. 1]) to yield cells with shorter pili (Table 2). However, the reduction in pilus length conferred by pORN138 was not as dramatic as that conferred by pORN127. Although the PilF$^-$ phenotype could be complemented by pORN138, the PilE$^+$ phenotype was not restored, suggesting that insertion mutations in *pilF* were indeed polar on *pilE* transcription (Table 2). We were unable to complement a chromosomal *pilE* lesion with plasmids constructed to contain only the *pilE* gene (e.g., pORN137; Table 2).

## DISCUSSION

The results presented here identify the protein product of the *pilE* gene that confers the ability of piliated cells to bind erythrocytes (8, 10). We also describe the location, function, and product of a second gene, *pilF*, lesions in which produce cells with abnormally long pili.

Lesions in *pilE* and *pilF* were obtained by λ Tn*5* mutagenesis of a subclone of the *pil* region encoding *pilE* and *pilF* and scoring for those cells having mutant plasmids that failed to complement a *pilE'-kan* lesion in the chromosome. Examination of the transcription-translation products of a number of noncomplementing mutant plasmids showed that lesions eliminating the production of either a 31-kDa protein or an 18-kDa protein abolished complementation. The 31-kDa gene product appeared to be the product of the *pilE* gene, because mutations eliminating this product clustered around the prototype *pilE* lesion made previously by restriction site mutagenesis (8). However, a second class of Tn*5* insertion mutations, those clustering at the right-hand end of the subcloned region, also abolished *pilE* complementation. These insertions were distinguished by the elimination of an 18-kDa protein. Mutants in this second class were termed *pilF* mutants. Insertion mutations in *pilF* appeared to be polar in that the 31-kDa product of the *pilE* gene was much reduced in *pilF*::Tn*5* mutants in minicells. This result was in contrast to results for insertion mutations in *pilE*, which did not noticeably affect the production of the 18-kDa *pilF* protein in minicells. A low level of expression of the *pilE* and *pilF* gene products in minicells was consistently noted and may reflect low-level in vivo production. Subcloning of the *pilE-pilF* region onto a variety of vectors did not noticeably improve the production of these proteins, although their presence was always apparent.

Our discovery that lesions in *pilF* produced, in addition to the PilE$^-$ phenotype, cells with longer pili (termed the PilF$^-$ phenotype) made possible further characterization of the nature of pilus assembly and allowed clarification of the effect that *pilF* insertion mutations had on *pilE* expression. The polar effect of the lesions in *pilF* on *pilE* was most clearly demonstrated by the complementation of the PilF$^-$ phenotype (conferred by a chromosomal insertion mutation in *pilF*) by a plasmid carrying only the *pilF* gene. Such complementation yielded cells with shorter pili but still having the PilE$^-$ phenotype. Had the chromosomal insertion mutation in *pilF* not been polar, one would expect that the PilE$^-$ phenotype would have been restored to wild type. The apparent relationship between the *pilE* and *pilF* genes at the transcriptional level may prove to be a clue to the role of the *pilE* gene product in pilus assembly and receptor binding.

The possibility remains that, in addition to polar effects of insertion mutations in *pilF*, the long pili possessed by the mutants preclude manifestation of the PilE$^+$ phenotype (ability to agglutinate guinea pig erythrocytes). However, this seems unlikely, since mutants producing long pili because of derepression of *pilA* transcription are capable of binding erythrocytes (13) (see below).

The increase in pilus length conferred by the lesions in *pilF* and the ability of a subclone containing the *pilF*-coding region to complement the *PilF*$^-$ phenotype argue strongly that the *pilF* gene product regulates pilus length. We believe that the easiest way for a single gene product to regulate pilus length would be for the *pilF* gene to encode a competitive inhibitor of piliation. That is, the *pilF* gene product would be a piluslike molecule that, once incorporated into the pilus, would stop the further addition of pilin subunits. Thus, on average, the length of a pilus would depend upon the ratio of pilin monomers available for polymerization to the number of molecules of the *pilF* gene product. One prediction we make from this is that hyperpiliated strains (i.e., strains having more pili because of a derepression of *pilA* transcription [13]) would also have longer pili, since the ratio of pilin to *pilF* gene product would go up. This indeed appears to be true (13). A more detailed prediction is that the *pilF* gene product and pilin would resemble each other at the

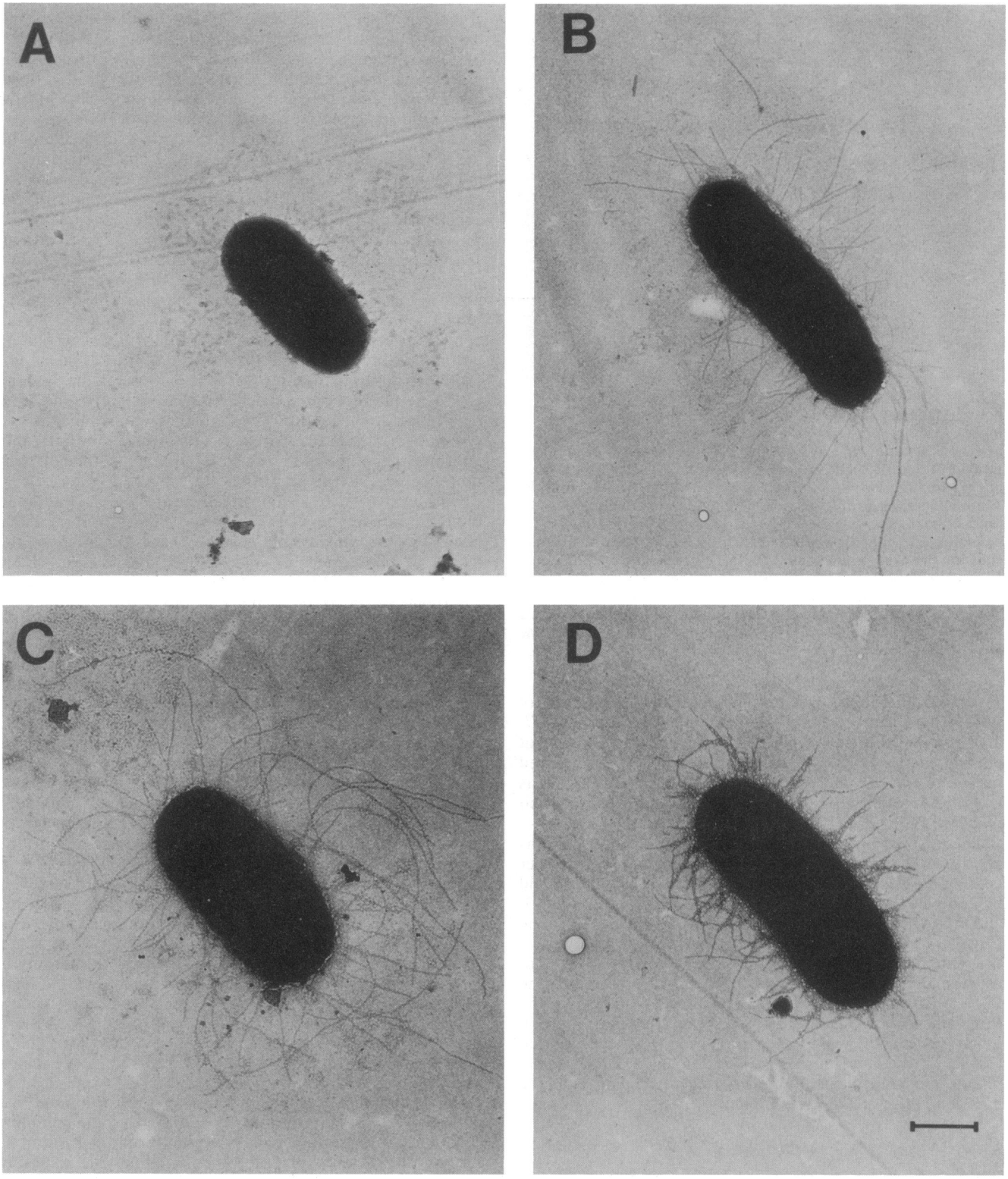


FIG. 3. Electron micrographs showing parental and *pilF* mutant cells and complementation of the PilF phenotype. (A) ORN147 (*pilA'-kan*). (B) ORN115 (*pilF*$^{+}$). (C) ORN140 (*pilF104*::Tn*5*). (D) ORN140(pORN127) (*pilE pilF*). Bar, 0.5 μm.

TABLE 2. Complementation analysis of strains ORN135 and ORN140[a]

| Plasmid | Genotype of plasmid[b] | Phenotype of strain containing the indicated plasmid[c]: ORN135 (*pilE105*::Tn*5*) | | ORN140 (*pilF104*::Tn*5*) | |
|---|---|---|---|---|---|
| | | PilE | PilF | PilE | PilF |
| pBR322 | | − | + | − | − |
| pRN2010 | | − | + | − | − |
| pORN127 | *pilE pilF* | + | + | + | + |
| pORN136 | *pilE pilF* | + | + | + | + |
| pORN134 | *pilE105*::Tn*5 pilF* | − | + | − | + |
| pORN135 | *pilE pilF*104::Tn*5* | − | + | − | − |
| pORN137 | *pilE* | − | + | − | − |
| PORN138 | *pilF* | − | + | − | + |

[a] Plasmids were introduced into the mutant strains by transformation, with selection for the appropriate antibiotic resistance marker on the vector. Piliation phenotypes were assessed as described in the text.

[b] pBR322 and pRN2010 are control plasmids.

[c] +, Positive phenotype (e.g., PilE$^+$ cells agglutinate guniea pig erythrocytes); −, negative phenotype (e.g., PilF$^-$ cells have abnormally long pili).

tertiary level. This prediction may be reflected in a similarity in amino acid sequence. In fact, minor components of the Pap pili (pili associated with *E. coli* strains causing pyelonephritis) required for receptor binding (6, 7, 11, 19) appear to have amino acid sequences similar to that of Pap pilin (S. Normark, personal communication).

The effect of a change in the ratio of pilin to *pilF* gene product on pilus length may indicate that pilus length is regulated differently than lambda tail length, in which a "tape measure" protein appears to determine the length of the lambda tails (5). However, a much more detailed examination of pilus length control is required to make meaningful comparisons. Nevertheless, the existence of the *pilF* gene indicates that the cell does indeed monitor pilus length.

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