

Characterization of *Escherichia coli* Type 1 Pilus Mutants with Altered Binding Specificities

SANDRA L. HARRIS, PATRICIA A. SPEARS, EDWARD A. HAVELL, TERRI S. HAMRICK,
JOHN R. HORTON, AND PAUL E. ORNDORFF*

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

Received 19 January 2001/Accepted 13 April 2001

PCR mutagenesis and a unique enrichment scheme were used to obtain two mutants, each with a single lesion in *fimH*, the chromosomal gene that encodes the adhesin protein (FimH) of *Escherichia coli* type 1 pili. These mutants were noteworthy in part because both were altered in the normal range of cell types bound by FimH. One mutation altered an amino acid at a site previously shown to be involved in temperature-dependent binding, and the other altered an amino acid lining the predicted FimH binding pocket.

Type 1 pili are filamentous proteinaceous appendages produced by many members of the family *Enterobacteriaceae*. In *Escherichia coli*, the biosynthesis and binding properties of these pili have been well studied (reviewed in reference 30). Pili are made principally of a repeating monomer, FimA, the product of the *fimA* gene (13), that is arrayed helically to form a hollow-cored fiber (2). There are at least three minor pilus proteins that are organized into structures seen on the ends of pili (12) and may also be present in the pilus fiber (22). One of these minor components, FimH, the product of the *fimH* gene, is the molecule that actually binds to mannose-containing receptors on eucaryotic cells (14). The precise nature of the affinity of FimH for mannose is unclear. However, it has been known for some time that different arrangements of mannose monomers and substituent groups affect the affinity of FimH for these substrates (5). It is also known that other pilus components, ostensibly interacting with FimH, also affect the specificity of the interaction of FimH with mannose (16).

In this study, we have identified *fimH* mutants with changes in binding specificity. One especially novel feature of both mutants is the ability to bind and agglutinate yeast cells at parental levels but failed to bind macrophages any better than a *fimH* insertion mutant.

Bacterial strains, plasmids, and growth conditions. The bacterial strains, which were all *E. coli* K-12 derivatives, and plasmids used are listed in Table 1. Media consisted of L broth, L agar (18), and maltose-tetrazolium agar (27). Antibiotic concentrations were as described previously (20).

Receptor specificity mutant isolation. Plasmid pORN163, containing the *fimH* gene flanked by the *EcoRI* and *SalI* restriction endonuclease sites, was used as a template for generating *fimH* PCR amplicons with a high proportion of mutations. Amplicons, obtained after 30 amplification cycles using a threefold-higher concentration of MgCl₂ than called for by standard PCR conditions (32), were ligated into *EcoRI*- and *SalI*-cleaved pBR322. This mutant amplicon pool was subsequently introduced (by transformation [15]) into a Δ *fim* strain

(ORN201) containing a plasmid, pORN307, carrying all the genes for fimbriation except *fimH*. In a typical experiment, approximately 8,000 transformant colonies were pooled and subjected to enrichment for receptor specificity mutants (defined as those mutants that bound guinea pig erythrocytes in the presence of either of two inhibitors: 250 mM fructose or 0.25 mM *p*-nitrophenyl α -D-manno-pyranoside [*N*-phenyl mannose]). Enrichment was accomplished by mixing 0.5 ml of the transformant pool (ca. 5×10^8 cells) with 100 μ l of settled guinea pig erythrocytes in phosphate-buffered saline (PBS) containing an inhibitor. After a 10-min room temperature incubation, erythrocytes were isolated by a 1-s centrifugation in a microcentrifuge and the supernatant was aspirated. The pellet was gently resuspended in 1.0 ml of PBS containing an inhibitor, and the erythrocytes were reisolated by centrifugation. After five more washing steps, the pelleted erythrocytes were resuspended in 1.0 ml of distilled water and diluted with 1.5 ml of L broth containing chloramphenicol and ampicillin, and the erythrocyte-bound population was expanded by overnight growth (with shaking) at 37°C. A second and third enrichment were typically employed. At the end of the procedure, broth cultures were streaked for colony isolation on L agar plates containing chloramphenicol and ampicillin. Individual colonies were screened for the ability to agglutinate erythrocytes in the presence of an inhibitor (10). Plasmids bearing candidate mutant *fimH* alleles were isolated (4) and reintroduced into strain ORN201 harboring pORN307 by transformation to confirm that the *fimH*-containing plasmid conferred the altered binding phenotype. One mutant per experiment was kept to ensure independent origin.

Characterization of the plasmid-borne alleles and introduction of *fimH* mutant alleles into the chromosome. Twenty-five plasmid-borne *fimH* specificity mutants were initially isolated (15 using fructose and 10 using *N*-phenyl mannose). Nineteen of the *fimH* alleles conferring the strongest phenotypes were completely sequenced employing the methods of Russell and Orndorff (24). Seventeen of the alleles were unique, but 13 of the 17 had more than one mutation in the *fimH* coding sequence. Six of the plasmid-borne mutant alleles that had the fewest mutations were cloned into pKAS32 and introduced

* Corresponding author. Mailing address: College of Veterinary Medicine, 4700 Hillsborough St., Raleigh, NC 27606. Phone: (919) 513-6207. Fax: (919) 513-6455. E-mail: Paul_Orndorff@ncsu.edu.

TABLE 1. Bacterial strains, phage, and plasmids used in the study

Strain, phage, or plasmid	Description	Source or reference
<i>E. coli</i>		
ORN115	<i>thr-1 leuB thi-1 Δ(argF-lac)U169 xyl-7 ara-13 mtl-2 gal-6 rpsL tonA2 supE44 pilG1 λ^r Pil⁺</i> (does not exhibit phase variation of piliation)	31
ORN174	<i>thr leu proA2 lacY1 galK his argE rpsL supE mtl xyl recBC sbcB tetR</i> (inserted ca. 200 bp 3' of the end of <i>fimH</i>) Pil ⁺	24
ORN178	ORN115 except <i>tetR</i> inserted ca. 200 bp 3' of the end of <i>fimH</i> , Pil ⁺	24
ORN201	<i>thr-1 leuB thi-1 Δ(argF-lac)U169 xyl-7 ara-13 mtl-2 gal-6 rpsL tonA2 supE44 pilG1 λ^r recA13 Δ(fimBEACDEFGH)</i>	9
ORN207	ORN174 except <i>fimH304::kan</i>	Linear transformation of ORN174 with <i>EcoRI</i> -digested pORN164 ^a
ORN208 ^b	ORN115 except <i>fimH304::kan</i> with adjacent <i>tetR</i> , Nal ^r	P1 transduction from ORN207 to ORN115 and selection of a nalidixic acid-resistant variant
ORN209	ORN115 except <i>fimH165</i> allele and adjacent <i>tetR</i> gene	This study
ORN210	ORN115 except <i>fimH166</i> allele and adjacent <i>tetR</i> gene	This study
Bacteriophage		
P1	<i>vir</i>	Laboratory collection
Plasmids		
pBR322	ColE1 Ap ^r Tc ^r	1
pKAS32 ^c	<i>oriR6K oriT rpsL</i> Ap ^r	28
pORN163 ^d	pBR322 <i>fimH</i> Ap ^r	Insertion of a 2-kb <i>PvuII</i> fragment containing <i>fimH</i> from pORN127 (17) into <i>BamHI</i> -cleaved pBR322
pORN307	pSH2 Δ <i>fimH</i> Cm ^r	8
pORN164	pORN304 <i>fimH304::kan</i> , has <i>kan</i> gene from Tn5 inserted in the <i>XhoI</i> site created as part of deletion in <i>fimH304</i>	Kan cassette inserted into <i>XhoI</i> site of pORN304 (8)
pORN165	pORN163 except <i>fimH165</i>	This study
pORN166	pORN163 except <i>fimH166</i>	This study

^a Linear transformation and P1 transduction have been previously described (21).

^b Strain ORN208 was used as the recipient in a mating and allelic exchange protocol (28) that introduced mutant *fimH* alleles into the chromosome, replacing the *fimH* insertion mutation.

^c Introduction of the *fimH* mutant alleles into the chromosome was accomplished by first subcloning each mutant *fimH* allele carried on the ca. 1.8-kb *SalI-EcoRI* fragment of pORN163 into *SalI-EcoRI*-digested pGEM11ZF (Promega) and then removing an *EcoRI-XbaI* fragment containing the *fimH* allele. (The new restriction endonuclease sites on the ends of the *fimH* alleles were contributed by the polylinker in pGEM11ZF.) *EcoRI-XbaI* fragments containing the *fimH* alleles were introduced into *EcoRI-XbaI*-cleaved pKAS32 and then introduced into the chromosome of strain ORN208 by allelic exchange (28). Refer to Fig. 1 for a diagram.

^d PCR-generated mutant *fimH* genes were obtained by amplification using primers 5'GGGTTATTGTCTCATGAGCG and 5'CCGATCATCGTCGCGTCCA flanking *EcoRI* and *SalI* sites in pBR322. PCR amplicons were digested with *EcoRI* and *SalI*, and the *SalI-EcoRI* fragments were isolated and ligated into *SalI-EcoRI*-cleaved pBR322. This ligation mixture was introduced into strain LE392 (19) by electroporation (25). Following electroporation, electroporant colonies were harvested and the plasmid DNA was extracted (4) and introduced into strain ORN201 harboring pORN307, as described in the text.

into the chromosome of ORN208 by allelic exchange (28) (diagrammed in Fig. 1).

Interestingly, only two of the six *fimH* mutant alleles (both identified by enrichment in 250 mM fructose) conferred an altered binding specificity phenotype when chromosomally located even though all six of the chromosomal alleles were confirmed to have sequences identical to the starting plasmid-encoded alleles (as indicated by the sequencing of PCR-generated amplicons of the entire *fimH* gene; UNC Automated DNA Sequencing Facility, Chapel Hill, N.C.). The rest of the mutants produced either the parental phenotype (one of six) or a null binding phenotype (three of six) with regard to yeast cell and erythrocyte agglutination.

Altered phenotypes conferred by the two chromosomal *fimH* mutant alleles. Both of the *fimH* chromosomal mutations that conferred altered binding specificities had single missense mutations predicted to cause an amino acid substitution within the first half of the mature FimH protein (alleles *fimH165* and *fimH166* from strains ORN209 and ORN210, respectively) (Table 2). Electron-microscopic examination using the methods of Hamrick et al. (8) revealed that strains ORN209 and ORN210 were similar to the parental strain in terms of pilus

number per cell and in pilus morphology (data not shown). Also, they were similar to the parent in terms of the ability to agglutinate yeast cells in simple titration tests (Table 2). In contrast to the parent, the mutants retained approximately 50% of this ability in the presence of either of the two inhibitors used to initially isolate the mutants (Table 2). Both mutants also showed a somewhat reduced level of FimH cross-reacting material in antiserum agglutination reactions and a reduced ability to agglutinate guinea pig erythrocytes, and they were unable to bind to macrophages any better than a *fimH* insertion mutant (Table 2).

Conclusions. Our results describe the utilization of mannose analogs to enrich mutants with different FimH binding specificities. The isolated mutants retained yeast cell binding activity in the presence of normally inhibitory mannose analogs and were additionally altered in the range of cell types they normally bound. Most striking was the complete loss of macrophage binding and the contrasting retention of parental levels of yeast cell agglutination.

Neither of the two single-site *fimH* mutations described here (*fimH165* and *fimH166*) have been previously reported. However, the lesion at amino acid position 142 (*fimH166*) has been

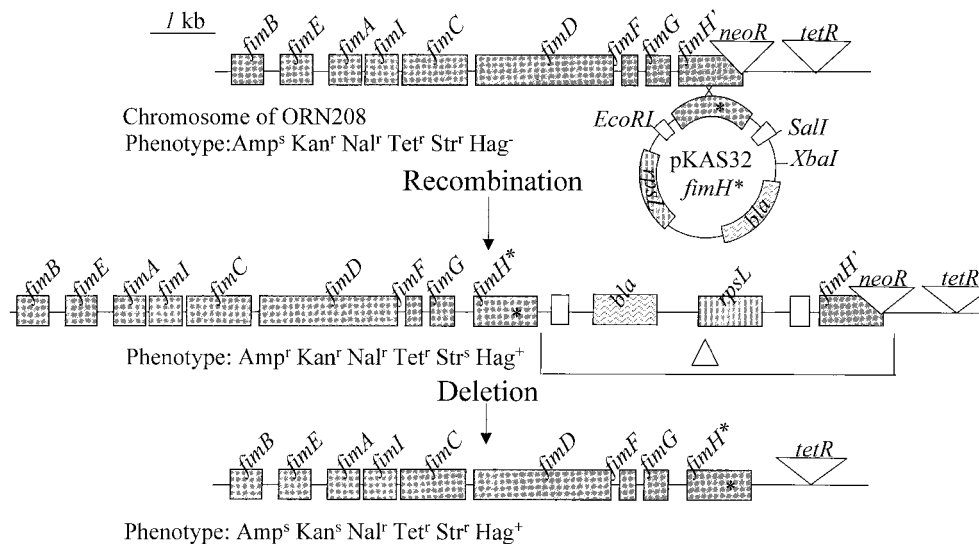


FIG. 1. Diagram of allelic exchange between a mutant *fimH* allele (*fimH**) carried on the nonreplicating vector pKAS32 and the *fim* region on the chromosome of strain ORN208. Following introduction of the plasmid by mating (28), recombinants were selected and scored for the phenotypic characteristics noted. Recombinants that had deletions (Δ) of the vector and the original *fimH* insertion mutation in ORN208 were identified by selection on streptomycin-containing agar and scored for the appropriate antibiotic and hemagglutination phenotype as indicated. Each *fimH* lesion introduced was more than 98% linked (by P1 transduction [18]) to the *tetR* insertion adjacent to *fimH*. Phenotypic designations denote sensitivity (s) or resistance (r) to antibiotics as follows: Nal, nalidixic acid; Kan, kanamycin; Tet, tetracycline; Amp, ampicillin; Str, streptomycin. Erythrocyte or yeast cell agglutination is collectively denoted phenotypically as Hag. Streptomycin sensitivity is conferred by the *rpsL* gene, kanamycin resistance is conferred by the *neoR* gene, ampicillin resistance is conferred by the *bla* gene, and tetracycline resistance is conferred by the *tetR* gene.

noted by Schembri et al. (26). Unfortunately, the allele described by Schembri et al. had an additional lesion. Consequently, the two alleles cannot be directly compared. Also, Schembri et al. utilized recombinant plasmid-borne *fimH* alleles. In the mutants we isolated, only one-third displayed the same phenotype when the plasmid-borne lesions were introduced into the chromosome. It is possible that overproduction

(or other effects) attendant with transcription from a recombinant plasmid, when combined with certain lesions, effected an altered binding phenotype that was not exhibited in the chromosome. We chose to examine only those *fimH* alleles whose mutant binding phenotypes were manifested in the chromosome.

Of the two chromosomal *fimH* mutants described here, the

TABLE 2. Summary of chromosomal *fimH* lesions and phenotypes associated with strains derived from the enrichment procedure^a

Strain	<i>fimH</i> allele	Lesion location (nucleotide ^b)	Amino acid change (position ^c)	Agglutination and binding reactions (% of value for parental strain) ^d				Yeast agglutination (% of titer) in PBS ^e	
				FimH antiserum agglutination	Macrophage binding	Erythrocyte agglutination	Yeast agglutination	0.25 mM <i>N</i> -phenyl mannose	250 mM fructose
ORN178	Parental	None	None	(100)	(100)	(100)	(100)	0 \pm 0	0 \pm 0
ORN208	<i>fimH308'-kan</i>	836–935 deletion	Δ (Arg–Gln) (258–279)	(0)	9.0 \pm 3.8	(0)	(0)	NA	NA
ORN209	<i>fimH165</i>	236 T→C	Leu→Pro (58)	58 \pm 22	7.8 \pm 3.8	44 \pm 9	100 \pm 20	37 \pm 5	41 \pm 2
ORN210	<i>fimH166</i>	488 T→C	Phe→Ser (142)	68 \pm 30	13.0 \pm 5.7	44 \pm 9	105 \pm 13	53 \pm 6	58 \pm 20

^a Presence of FimH in mutant strains was determined by immunological and functional criteria. Polyclonal antiserum to a LacZ-FimH fusion protein was employed in bacterial agglutination reactions using serially diluted antiserum (8). Functional criteria for the presence of FimH consisted of measuring the ability of strains to agglutinate guinea pig erythrocytes and yeast as described by Hamrick et al. (8) with the following modifications. Flat-bottomed 96-well plates were used, and the bacteria and erythrocytes were gently mixed in order to effect agglutination, which took place within a few minutes. Yeast agglutination reactions employed 25 mg of baker's yeast/ml in PBS in place of erythrocytes.

^b Refers to the position for *E. coli* K-12 *fimH* in GenBank. Our parental allele, while not originally from *E. coli* K-12, has an identical sequence.

^c Follows the convention of Sokurenko et al. (29) where the 279 amino acids of the mature FimH protein are numbered sequentially from 1 to 279.

^d In the case of agglutination reactions, the \log_2 of the highest bacterial or antiserum dilution in which agglutination was witnessed was calculated so as to produce a linear scale of agglutination values which were normalized to the value for the parental strain and expressed as a percentage. Values in parentheses indicate defined values. The insertion mutant (strain ORN208) showed no agglutination titer under any conditions. All mutant values were statistically significantly different from the parental values but not from each other, as determined in the two-tailed unpaired *t* test as previously described (8). The assay for the ability of type 1 pilated *E. coli* to bind BALB/c mouse resident peritoneal macrophages has been described previously (8). P1 transduction was used to introduce the appropriate *fimH* mutant alleles into the genetically marked strains used in the assay. Values are expressed as means \pm standard deviations.

^e The \log_2 of the highest dilution of bacteria in which agglutination was witnessed in the presence of an inhibitor was calculated so as to produce a linear scale of agglutination values, which were normalized to the agglutination titer in the absence of inhibitor and expressed as a percentage. Both inhibitors completely blocked erythrocyte and yeast agglutination by the parental strain even at the lowest dilution of bacteria. All mutant values were statistically significantly different from the parental values but not from each other. Methods for determining the statistical significance of mean differences were as previously described (8). NA, not applicable. Values are expressed as means \pm standard deviations.

one with the *fimH165* allele (a Leu→Pro substitution at position 58 of FimH) was of particular interest because a mutant with a lesion at the same nucleotide (conferring a Leu→Arg change) has been noted to confer a temperature-dependent binding phenotype (8). The present Leu→Pro substitution effected a complete absence of macrophage binding but the mutant retained full ability to agglutinate yeast and partial ability to agglutinate erythrocytes. In contrast, the Leu→Arg mutant retains macrophage binding ability at the restrictive temperature (albeit with an altered specificity) but erythrocyte binding ability is lost (8). The previous and present results indicate that the type of amino acid at position 58 influences two important traits: (i) the conditions under which FimH is active and (ii) the specificity of that interaction. The lesion we found at amino acid position 142 (*fimH166*) changes an amino acid predicted to line the FimH binding pocket (3). The location of this lesion would appear to make a good deal of structural sense (i.e., a change in receptor specificity attributable to an amino acid lining the binding pocket).

Quite a variety of eucaryotic cells bind type 1 piliated *E. coli* via FimH. However, the FimH receptor on each eucaryotic cell type differs (6, 7, 11). Consequently, FimH mutants of the type reported here (i.e., those that have retained the affinity for one cell type but not another) may be particularly useful in better understanding issues such as bacterial tissue tropism (23) and intra- and intercellular signaling initiated by the interaction of FimH with particular types of eucaryotic cells (11).

Nucleotide sequence accession numbers. The *fimH165* and *fimH166* alleles have been submitted to GenBank. They have been given the following accession numbers: *fimH165*, AF306535; and *fimH166*, AF306536.

We thank Craig Altier for a critical reading of the manuscript.

This work was supported by a grant from the National Institutes of Health (AI22223).

REFERENCES

- Bolivar, F. 1978. Construction and characterization of new cloning vehicles. III. Derivatives of plasmid pBR322 carrying unique *EcoRI* sites for selection of *EcoRI*-generated recombinant molecules. *Gene* **4**:121–136.
- Brinton, C. C., Jr. 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.
- Choudhury, D., A. Thompson, V. Stojanoff, S. Langermann, J. Pinkner, S. J. Hultgren, and S. D. Knight. 1999. X-ray structure of the FimC-FimH chaperone-adhesin complex from uropathogenic *Escherichia coli*. *Science* **285**:1061–1066.
- Davis, R. W., D. Botstein, and J. R. Roth. 1980. Advanced bacterial genetics, p. 90. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Firon, N., S. Ashkenazi, D. Mirelman, I. Ofek, and N. Sharon. 1987. Aromatic alpha-glycosides of mannose are powerful inhibitors of the adherence of type 1 fimbriated *Escherichia coli* to yeast and intestinal epithelial cells. *Infect. Immun.* **55**:472–476.
- Gbarah, A., C., G. Gahmberg, I. Ofek, U. Jacobi, and N. Sharon. 1991. Identification of the leukocyte adhesion molecules CD11 and CD18 as receptors for type 1-fimbriated (mannose-specific) *Escherichia coli*. *Infect. Immun.* **59**:4524–4530.
- Giampapa, C. S., S. N. Abraham, T. M. Chiang, and E. H. Beachey. 1988. Isolation and characterization of a receptor for type 1 fimbriae of *Escherichia coli* from guinea pig erythrocytes. *J. Biol. Chem.* **263**:5362–5367.
- Hamrick, T. S., S. L. Harris, P. A. Spears, E. A. Havell, J. R. Horton, P. W. Russell, and P. E. Orndorff. 2000. Genetic characterization of *Escherichia coli* type 1 pilus adhesin mutants and identification of a novel binding phenotype. *J. Bacteriol.* **182**:4012–4021.
- Hamrick, T. S., E. A. Havell, J. R. Horton, and P. E. Orndorff. 2000. Host and bacterial factors involved in the innate ability of mouse macrophages to eliminate internalized unopsonized *Escherichia coli*. *Infect. Immun.* **68**:125–132.
- Harris, S. L., D. A. Elliott, M. C. Blake, L. M. Must, M. Messenger, and P. E. Orndorff. 1990. Isolation and characterization of mutants with lesions affecting pellicle formation and erythrocyte agglutination by type 1 piliated *Escherichia coli*. *J. Bacteriol.* **172**:6411–6418.
- Hedlund, M., B. Frendeus, C. Wachtler, L. Hang, H. Fischer, and C. Svanborg. 2001. Type 1 fimbriae deliver an LPS- and TLR4-dependent activation signal to CD14-negative cells. *Mol. Microbiol.* **39**:542–552.
- Jones, C. H., J. S. Pinkner, R. Roth, J. Heuser, A. V. Nicholes, S. N. Abraham, and S. J. Hultgren. 1995. FimH adhesin of type 1 pili is assembled into a fibrillar tip structure in the Enterobacteriaceae. *Proc. Natl. Acad. Sci. USA* **92**:2081–2085.
- Klemm, P. 1984. The *fimA* gene encoding the type-1 fimbrial subunit of *Escherichia coli*. Nucleotide sequence and primary structure of the protein. *Eur. J. Biochem.* **143**:395–399.
- Krogfelt, K. A., H. Bergmans, and P. Klemm. 1990. Direct evidence that the FimH protein is the mannose-specific adhesin of *Escherichia coli* type 1 fimbriae. *Infect. Immun.* **58**:1995–1998.
- Lederberg, E. M., and S. N. Cohen. 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. *J. Bacteriol.* **119**:1072–1074.
- Madison, B., I. Ofek, S. Clegg, and S. N. Abraham. 1994. Type 1 fimbrial shafts of *Escherichia coli* and *Klebsiella pneumoniae* influence sugar-binding specificities of their FimH adhesins. *Infect. Immun.* **62**:843–848.
- Maurer, L., and P. E. Orndorff. 1985. A new locus, *pilE*, required for the binding of type 1 piliated *Escherichia coli* to erythrocytes. *FEMS Microbiol. Lett.* **30**:59–66.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Murray, N. E., W. J. Brammer, and K. Murray. 1977. Lambdoid phages that simplify the recovery of *in vitro* recombinants. *Mol. Gen. Genet.* **150**:53–61.
- Orndorff, P. E., and S. Falkow. 1984. Organization and expression of genes responsible for type 1 piliation in *Escherichia coli*. *J. Bacteriol.* **159**:736–744.
- Orndorff, P. E., P. A. Spears, D. Schauer, and S. Falkow. 1985. Two modes of control of *pilA*, the gene encoding type 1 pilin in *Escherichia coli*. *J. Bacteriol.* **164**:321–330.
- Ponniah, S., R. O. Endres, D. L. Hasty, and S. N. Abraham. 1991. Fragmentation of *Escherichia coli* type 1 fimbriae exposes cryptic D-mannose-binding sites. *J. Bacteriol.* **173**:4195–4202.
- Pouttu, R., T. Paustinen, R. Virkola, J. Hacker, P. Klemm, and T. K. Korhonen. 1999. Amino acid residue Ala-62 in the FimH fimbrial adhesin is critical for the adhesiveness of meningitis-associated *Escherichia coli* to collagens. *Mol. Microbiol.* **31**:1747–1757.
- Russell, P. W., and P. E. Orndorff. 1992. Lesions in two *Escherichia coli* type 1 pilus genes alter pilus number and length without affecting receptor binding. *J. Bacteriol.* **174**:5923–5935.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Schembri, M. A., E. V. Sokurenko, and P. Klemm. 2000. Functional flexibility of the FimH adhesin: insights from a random mutant library. *Infect. Immun.* **68**:2638–2646.
- Silhavy, T. J., M. L. Berman, and L. W. Enquist. 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Skorupski, K., and R. K. Taylor. 1996. Positive selection vectors for allelic exchange. *Gene* **169**:47–52.
- Sokurenko, E. V., H. S. Courtney, D. E. Ohman, P. Klemm, and D. L. Hasty. 1994. FimH family of type 1 fimbrial adhesins: functional heterogeneity due to minor sequence variations among *fimH* genes. *J. Bacteriol.* **176**:748–755.
- Soto, G. E., and S. J. Hultgren. 1999. Bacterial adhesins: common themes and variations in architecture and assembly. *J. Bacteriol.* **181**:1059–1071.
- Spears, P. A., D. Schauer, and P. E. Orndorff. 1986. Metastable regulation of type 1 piliation in *Escherichia coli* and isolation and characterization of a phenotypically stable mutant. *J. Bacteriol.* **168**:179–185.
- Zhou, Y., X. Zhang, and R. H. Ebright. 1991. Random mutagenesis of genome-sized DNA molecules by use of PCR with Taq DNA polymerase. *Nucleic Acids Res.* **19**:6052.