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Type 1 fimbriae mutants of *Escherichia coli* K12: characterization of recognized afimbriate strains and construction of new *fim* deletion mutants

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Summary

We have used Southern hybridization analysis to characterize the extent of *fim* homology in recognized type 1 fimbriae mutants of *Escherichia coli* K12, including strains HB101, P678-54, and VL584. We have found extensive homology in strain HB101, and confirm that P678-54 lacks the majority of *fim* DNA. Strain VL584 contains a deletion of the entire *fim* region. We have used a new allelic exchange procedure to generate novel *fim* deletion derivatives of strains MG1655, MM294, and YMC9. To increase the utility of the new deletion strains we also isolated *recA* derivatives of each mutant. These strains facilitate the isolation, characterization, and manipulation of cloned fimbriae genes from diverse sources.

Introduction

The construction of mutants is an essential element in the genetic analysis of microbial phenotypes and in the manipulation of cloned genes. The mutants that contain a deletion of the entire region of interest, being those that are least capable of reverting to wild type, are often the most useful for cloned-gene manipulation and genetic analysis. Thus, strains of *Escherichia coli* are often selected as cloning hosts, in part by virtue of the fact that they contain chromosomal deletions in the genes that are to be cloned and expressed.

We are particularly interested in the genetic regulation in *E. coli* of type 1 fimbriae, which are peritrichously arranged organelles that are important in bacterial adherence and colonization. The purpose of this communication is two-fold: first, we describe the Southern hybridization and partial genetic analysis of three known Fim$^-$ mutants of *E. coli* K12, i.e. HB101 (Boyer and Roulland-Dussoix, 1969), P678-54 (Alder *et al.*, 1967), and VL584 (Freitag *et al.*, 1985). Strain HB101, lacking the HsdR restriction enzyme and being phenotypically afimbriate, has been used widely in the cloning and subsequent study of type 1 (Clegg, 1982; Purcell and Clegg, 1983; Orndorff and Falkow, 1984; Clegg *et al.*, 1985; Klemm *et al.*, 1985; Orndorff *et al.*, 1985; Klemm, 1986) and other classes of fimbriae (Marrs *et al.*, 1985; Morrissey and Dougan, 1986; Ott *et al.*, 1986; Pawelzik *et al.*, 1988). However, this strain also lacks the HsdM methylase, and consequently transfer of plasmid DNA from HB101 to *hsdR*$^+$ strains is inefficient. Moreover, HB101 appears to carry chromosomal markers capable of complementing defects in some of the cloned fimbriae genes (Pallesen *et al.*, 1989). Here we show that HB101 contains considerable homology to the type 1 fimbriae genes. We also confirm that P678-54, another strain employed in the analysis of fimbriae (Hull *et al.*, 1981; Normark *et al.*, 1983; Orndorff and Falkow, 1984; Orndorff *et al.*, 1985), lacks the majority of *fim* sequences (Moseley *et al.*, 1980). Nevertheless, both Southern hybridization analysis and genetic complementation studies suggest that P678-54 retains a copy of the important regulatory gene, *fimB* (Klemm, 1986). In addition, we report that strain VL584, constructed in this laboratory as a derivative of strain CSH50 (Miller, 1972), contains a deletion of all the known type 1 fimbriae genes.

The second purpose of this communication, in the light of our studies of the recognized afimbriate mutants, and in response to the need for *fim*-deleted, *hsdR*$^-$ *hsdM*$^+$ host strains in our and other laboratories, is the construction of new Δ*fim*, Δ*recA* mutants of the *E. coli* K12 strain MM294 (Meselson and Yuan, 1968) and its Δ*lac* derivative, YMC9 (Backman *et al.*, 1981). We have also constructed a deletion mutant of the wild-type strain MG1655 (Guyer *et al.*, 1980). This mutant, because it has undergone comparatively little manipulation, and because it contains a defined deletion of the type 1 fimbriae genes, should provide an improved host for studies of plasmid-based fimbriae expression.

Results

Southern hybridization analysis of afimbriate strains

Chromosomal DNA isolated from strains P678-54 (kindly supplied by B. Bachmann as CGSC4928), HB101 (from

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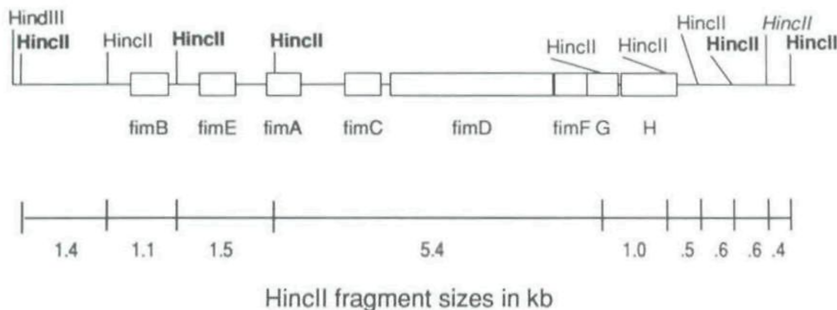

Probe A (HindIII-SalI)

Probe B (HincII-HincII)

Fig. 1. Genetic and physical organization of the *fim* region of *E. coli* K12. The positions of *Hin*dIII, *Hpa*I, *Hin*cII and *Sal*I restriction endonuclease sites, together with the *Hin*cII restriction fragment sizes, are shown. The subset of *Hin*cII sites that are also cut by *Hpa*I are emphasized in bold type. The unique *Sal*I site is indicated by italics (*Hin*cII), and is the second site from the right. The extent of probes A and B is also indicated. Probe DNA comprises gel-purified restriction endonuclease fragments derived from the cloned type 1 fimbriae genes of strain MG1655 and was labelled using the multiprime kit supplied by Amersham.

our own and P. Klemm's laboratory), VL584, and MG1655 (CGSC6300, wild-type fimbriate strain) were digested with either *Hpa*I or *Hin*cII and examined using Southern hybridization. The organization of the wild-type *fim* region, showing the positions of relevant restriction endonuclease sites, is shown in Fig. 1. The positions of most restriction endonuclease sites were originally determined by Klemm (Klemm *et al.*, 1985) and recently confirmed and extended in our laboratory with independently cloned fimbriae genes (our unpublished data). Duplicate Southern blots were prepared, and each blot was hybridized to one of two *fim* DNA probes. Probe A, a 12 kbp *Hin*dIII–*Sal*I fragment, encompasses the entire *fim* gene cluster, and probe B, a 1.4 kbp *Hin*cII fragment, includes sequences upstream of *fimB* (Fig. 1).

An *Hpa*I digest of chromosomal DNA isolated from MG1655 produces four bands of 7.3, 2.5, 1.5 and 0.9 kbp that hybridize with probe A (Fig. 2, lane 4). Probe B hybridizes with the 2.5 kbp fragment only; thus, this band encompasses the region upstream of, and including, the *fimB* gene (data not shown). Digestion of DNA isolated from P678-54 with *Hpa*I produces a single 2.5 kbp fragment that hybridizes to both probes, suggesting that this strain may contain the *fimB* gene (Fig. 2, lane 3). To investigate this hypothesis, we carried out additional hybridization studies with chromosomal DNA digested with *Hin*cII. Hybridization of *Hin*cII-digested MG1655 DNA to probe A led to the detection of several fragments, including sequences of 1.4 kbp and 1.1 kbp, that include the region upstream of, and including, *fimB*, respectively (Figs 1 and 4). Hybridization of the *Hin*cII-digested MG1655 DNA with probe B detected the 1.4 kbp band only (Fig. 4), verifying the identity of this fragment. As predicted, fragments of 1.4 kbp and 1.1 kbp are also seen in strain P678-54 (data not shown). We have also determined, using a *fimB*-specific probe (data not shown), that the 1.1 kbp *Hin*cII fragment of strain P678-54 includes at least part of the *fimB* gene. These data confirm that P678-54 contains a deletion of most of the *fim* gene cluster (Moseley *et al.*, 1980), but suggest that it may retain a copy of *fimB*.

Hybridization of DNA isolated from our laboratory stock of HB101 to probe A shows that this strain contains

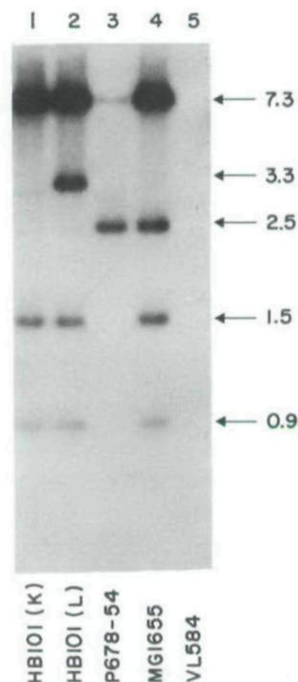

Fig. 2. Southern hybridization analysis of *fim* homology in afimbriate mutants, HB101, P678-54, and VL584, and fimbriate strain MG1655. Chromosomal DNA from different stocks of HB101, (K, Klemm; L, our own laboratory) was included in the study for comparison. DNA isolated from the indicated strain was digested with *Hpa*I, separated on a 1% agarose gel, and simultaneously blotted to nitrocellulose to produce duplicate filters. Hybridizations were carried out at high stringency (Maniatis *et al.*, 1982) using (A) the entire *fim* region (probe A, Fig. 1), or (B) a 1.4 kbp *Hin*cII fragment (probe B, Fig. 1) (not shown). The sizes of restriction fragments are indicated in kbp.

extensive *fim* homology (Fig. 2, lane 2). Digestion of the DNA with *Hpa*I produces three bands of 7.3, 1.5, and 0.9 kbp that co-migrate with the MG1655 sequences, and a fourth band of approximately 3.3 kbp (Fig. 2). Since the larger (3.3 kbp) fragment hybridizes with probe B, we suggest that our laboratory stock of HB101 contains an insertion of approximately 0.8 kbp in the *fimB* region (data not shown). Surprisingly, the second stock of HB101 contains a deletion in the *fimB* region; we detected no signal when chromosomal DNA was hybridized to probe B (data not shown). Finally, we were unable to detect any homology between the *fim* probes and chromosomal DNA isolated from strain VL584, showing that this strain contains a deletion of the entire *fim* region (Fig. 2, lane 5).

We have shown previously that the oscillating inversion of a 314 bp chromosomal element controls fimbrial phase variation (Abraham *et al.*, 1985). Klemm and colleagues have determined that *fimB* and *fimE* influence this inversion and have suggested that their products may in fact be site-specific DNA recombinases (Klemm, 1986; Pallesen *et al.*, 1989). In line with the Klemm hypothesis, strains lacking both *fimB* and *fimE* should not support recombination of the invertible element. To investigate the genetic content of strains HB101, P678-54, and VL584 further, we tested whether the invertible element, carried on a plasmid, rearranged in the appropriate site-specific manner in each strain. Accordingly, each strain was transformed with a plasmid containing the *fim* invertible element, but lacking both *fimB* and *fimE* (our unpublished data), and the orientation of the invertible element was determined by restriction endonuclease digestion and Southern hybridization analysis (data not shown). We found that although strain P678-54 is capable of supporting inversion of a plasmid-borne *fim* invertible element, strains VL584 and HB101 (from our laboratory) were not.

Construction of fim *deletion strains by allelic exchange*

We have used allelic exchange and P1 transduction to generate afimbriate *recA* mutants of *E. coli* K12 strains MG1655, MM294, and YMC9. The Δ*fim* mutants were constructed using allelic exchange; *recA* mutants of each Δ*fim* derivative were then isolated using P1 transduction. The allelic exchange procedure, which is described in detail in the accompanying paper (Blomfield *et al.*, 1991), employs a temperature-sensitive cloning vector (Hamilton *et al.*, 1989). Used in conjunction with a specially constructed intermediate strain that contains a *sacB*–Neo^R cassette (counterselectable and selectable markers, respectively) replacing part of the targeted chromosomal sequences, cloned DNA is exchanged for the corresponding chromosomal region in three steps. In the first step, plasmid integrate derivatives of the intermediate strain are selected at the non-permissive temperature for plasmid replication (42°C). Following a period of growth at the elevated temperature (the second step), bacteria are plated onto sucrose agar. Since the expression of *sacB* is lethal in the presence of sucrose, this procedure permits selection of recombinant bacteria that have both excised and cured the chromosomal markers.

To construct the Δ*fim* mutants, we employed the *sacB*-containing relative of MG1655, strain AAEC064, or similar derivatives of strains MM294 or YMC9, in conjunction with the temperature-sensitive plasmid, pIB310. Plasmid pIB310 contains an extensive deletion of *fim* DNA, but retains sequences that flank the known *fim* genes. The plasmid contains 1.4 kbp of DNA corresponding to the region situated upstream of *fimB*, extending from the *Hin*dIII site to an *Eco*0109 site, and 0.6 kbp normally situated downstream of *fimH* extending from an *Hpa*I site to the *Sal*I site (Fig. 3).

The construction of the *sacB* derivative of strain MG1655, AAEC064, is described in the accompanying paper (Blomfield *et al.*, 1991). In constructing similar derivatives of MM294 and YMC9, we transferred the *sacB* genes from AAEC064 using allelic exchange. Use of this approach, rather than P1 transduction, ensured that the intermediate derivatives did not acquire the closely linked wild-type *hsdR* region from strain AAEC064. We moved the *sacB*–Neo^R cassette situated on the temperature-sensitive plasmid pIB230 into the chromosome of MM294 and YMC9, essentially as described previously (Blomfield *et al.*, 1991). Plasmid pIB230 was first constructed *in vivo* by exchange of the *sacB*–Neo^R cassette contained in strain AAEC064 for the wild-type *fim* sequences situated on plasmid pIB308.

We transferred the *fim* deletion from plasmid pIB310 into the chromosomes of the intermediate strains using the protocol outlined above; Fig. 3 illustrates the expected allelic exchange. As predicted, the mutants were phenotypically afimbriate, failing to react with a type 1 fimbriae-specific monoclonal antibody (Eisenstein *et al.*, 1983) or to agglutinate yeast cells (Duguid and Gillies, 1957). To confirm that each strain had acquired the correct chromosomal structure, one sucrose-resistant isolate of each intermediate strain was examined by Southern hybridization analysis (Fig. 4). Chromosomal DNA isolated from the original parent strains (MG1655, MM294, and YMC9), the intermediate derivatives (AAEC064, AAEC160, and AAEC162), and the corresponding Δ*fim* derivatives (AAEC072, AAEC173, and AAEC175) were digested with *Hin*cII and blotted onto duplicate nitrocellulose filters following separation on a 1% agarose gel. Irrespective of which probe was used (probe A or B, Fig. 1), we detected an identical pattern of bands in each deletion mutant (Fig. 4). In each case, only a single prominent band of the expected 2.0 kbp size was seen.

To increase the utility of the Δ*fim* mutants for later

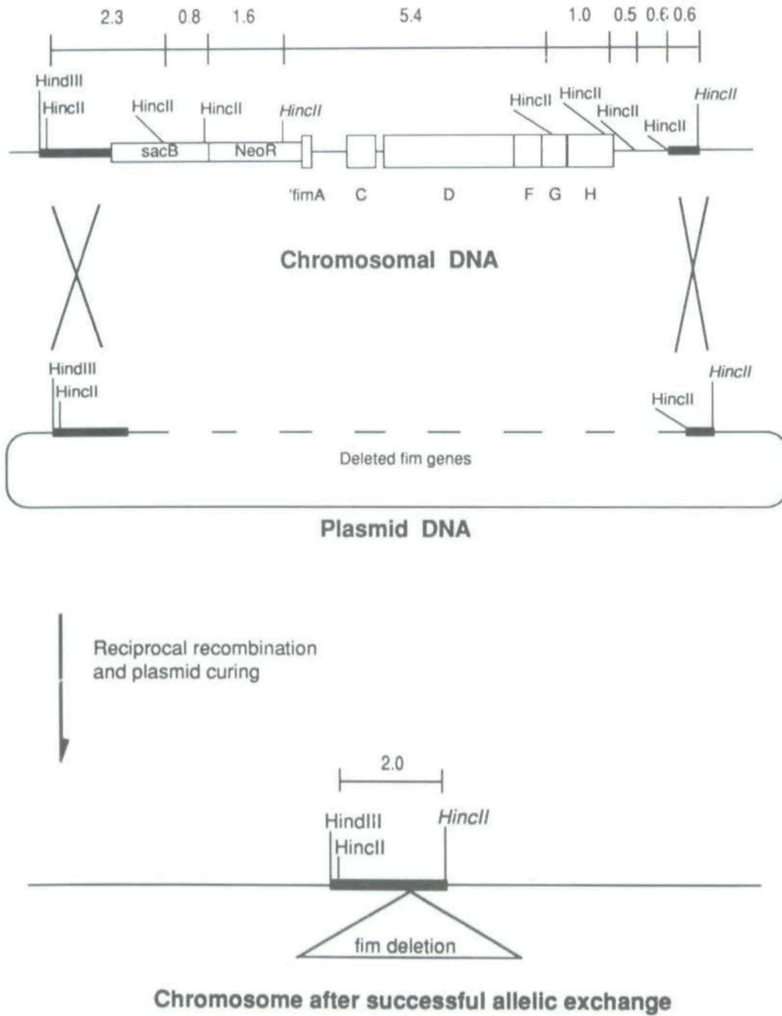

Fig. 3. Homologous recombination generates *fim* deletion mutants by allelic exchange. The physical and genetic organization of the intermediate strains (AAEC064, AAEC160, and AAEC162), showing the positions of *Hin*dIII, *Hin*cII and *Sal*I restriction endonuclease sites, together with the *Hin*cII restriction fragment sizes, are shown. Homologous sequences (shaded) shared by the temperature-sensitive plasmid pIB310 and the intermediate strains are indicated. The subset of *Hin*cII sites that are also cut by *Sal*I are indicated by italics; these sites are within the chromosomal Neo^R gene and at the rightmost part of the chromosomal DNA shown (both before and after recombination) and of the plasmid DNA insert.

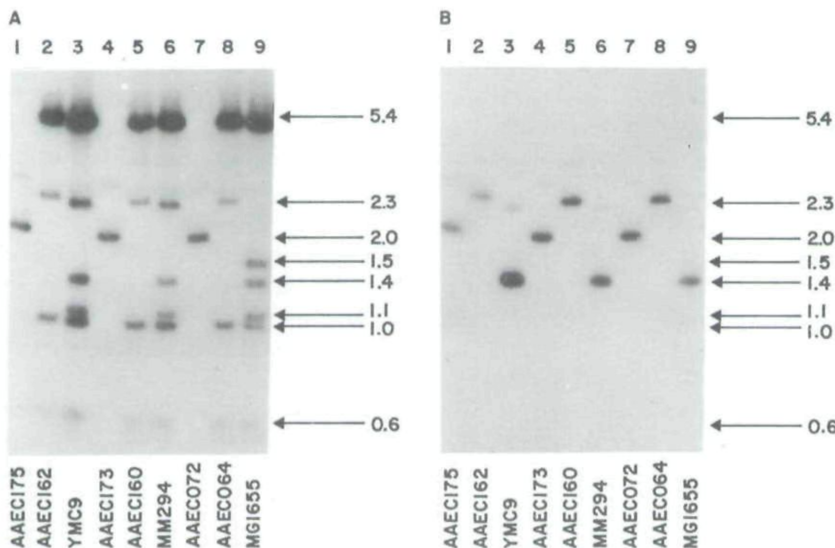

Fig. 4. Southern hybridization analysis of parent (MG1655, MM294, and YMC9), intermediate (AAEC064, AAEC160, and AAEC162) and *fim*-deletion (AAEC072, AAEC172, and AAEC175) strains. Chromosomal DNA isolated from the indicated strain was digested with *Hin*cII, separated on a 1% agarose gel and simultaneously blotted to nitrocellulose to produce duplicate filters. Hybridizations were carried out at high stringency (Maniatis *et al.*, 1982) using (A) the entire *fim* region (probe A, Fig. 1) or (B) a 1.4 kbp *Hin*cII fragment (probe B, Fig. 1). The sizes of restriction fragments are indicated in kbp. Note that strains MM294 and YMC9 lack the 1.5 kbp fragment detected in MG1655 when probe A is used. These strains contain a fragment of 2.3 kbp instead, suggesting that they may contain an insertion of 0.8 kbp in the *fimE* region.

genetic studies, we next isolated *recA* derivatives of each strain. Bacteriophage P1, grown on strain JC10240 (Csonka and Clark, 1979), was used to transduce the *recA* deletion and the closely linked copy of Tn*10* into each Δ*fim* mutant. Tetracycline-resistant transductants of AAEC072, AAEC173, and AAEC175, termed AAEC078, AAEC177, and AAEC179, respectively, were screened for increased sensitivity to ultraviolet irradiation to confirm acquisition of the *recA* deletion. Finally, we isolated tetracycline-sensitive derivatives of each strain by selecting mutants resistant to quinaldic acid (Bochner *et al.*, 1980). Tetracycline-sensitive mutants of strains AAEC078, AAEC177, and AAEC179 were named AAEC191, AAEC185, and AAEC189, respectively.

Discussion

In this study we have found that two afimbriate *E. coli* strains, HB101 and P678-54, commonly used in the cloning and genetic analysis of fimbriae genes, contain segments of *fim* DNA. Samples of strain HB101 from our laboratory and from that of Klemm (Klemm *et al.*, 1985) each contain considerable *fim* homology. Nevertheless, they differ in their hybridization patterns to the *fim* probes. In contrast, strain VL584 was found to contain an extensive deletion that includes, and goes beyond, the entire *fim* region. The hybridization analysis of strain P678-54, together with our genetic complementation data, suggest that the *fim* deletion in this strain extends from a point downstream of *fimB* to a position beyond the end of the *fimH* gene. Thus strain P678-54 seems to contain an active copy of *fimB*.

The simplest interpretation of our hybridization analysis of HB101 is that the two examined samples of this strain, although different, each contain lesions in the *fimB* region. Genetic complementation analysis of HB101 from our own laboratory suggests that this strain lacks both *fimB* and *fimE*; the strain is unable to support DNA inversion on a plasmid substrate. In sharp contrast, Klemm and colleagues have observed evidence of genetic complementation of the invertible element in their HB101 strain (Pallesen *et al.*, 1989). Since *fimE* mutants are fimbriate (Orndorff and Falkow, 1984), and since neither sample of HB101 produces fimbriae when transformed with the cloned *fimB* gene (data not shown), we suggest that both of these strains contain additional lesions. Given the contradictory complementation data described above, together with our finding of an active *fimB* in P678-54, we recommend that some prior observations concerning *fim* regulation should be repeated in a *fim*-deleted genetic background.

In view of the problems noted above, we felt compelled to construct new *fim* mutants that would serve as better hosts for future studies of fimbriae regulation. By introducing a deletion into cloned fimbriae genes *in vitro* and then transferring the construction into the chromosome by allelic exchange, we have constructed a panel of Δ*fim* mutants that should serve a variety of needs. These strains have several useful characteristics, in addition to containing a defined and complete deletion of all the known type 1 fimbriae genes. Several constructs will be especially appropriate for cloning purposes, whereas another, discussed below, is as close to the K12 wild type as is practicable and will be particularly useful in studies of genetic regulation. Thus, for gene cloning, the Δ*fim* mutants of MM294 (strain AAEC185) and of its Δ*lac* derivative, YMC9 (strain AAEC189), are *recA*$^-$ *endA*$^-$ and lack the *hsd* restriction endonuclease, but, unlike HB101, retain the *hsd* methylase. As such, they are efficiently transformed and produce excellent yields of plasmid DNA. Since plasmid DNA isolated from these strains is appropriately methylated, it efficiently transforms *hsdR*$^+$ strains such as MG1655 (data not shown). Moreover, since these strains lack the HsdR restriction enzyme, they should provide useful hosts for cloning various fimbriae genes from many sources. Likewise, strain AAEC189, containing a deletion of the *lac* genes, will be useful for constructing and manipulating *fim–lac* fusions, as we have already found (data not shown).

The characteristics displayed by strains AAEC185 and AAEC189, although preferable in strains used to clone and manipulate DNA, are less important for plasmid-based studies of gene control. In such a situation it is preferable to employ a strain that resembles the wild type as closely as possible. To fulfil this requirement, we constructed a Δ*fim* derivative of our K12 wild-type strain MG1655, a strain that has undergone comparatively little manipulation. An additional benefit of using MG1655 is that, as the host for an extensive collection of drug resistance markers covering the entire chromosome (Singer *et al.*, 1989), genetic manipulation of this strain (e.g. by P1 transduction) is comparatively easy. In view of these advantages, and the fact that the *fim* deletion in AAEC072 is so well defined, we consider this MG1655 derivative a more desirable host than VL584.

The novel and isogenic *fim* deletion mutants described in this communication should be useful in a variety of studies involving fimbriae regulation. For example, they should help to clarify the relationship between type 1 fimbriae, colony morphology, and Flu variation (Diderichsen, 1980). Variants of *flu* are distinguished by differences in colony morphology and auto-agglutination; *flu*$^-$ bacteria, which auto-agglutinate during growth in liquid culture, form larger rougher colonies than *flu*$^+$ cells, which do not auto-agglutinate. Variation of the *flu* phenotype apparently correlates with the expression of type 1 fimbriae, in that *flu*$^+$ cells are fimbriate, whereas *flu*$^-$ cells are not. We are currently examining our Δ*fim* derivatives to

assess which, if any, of the characteristics associated with *flu* variation are determined directly by the presence of type 1 fimbriae. In addition, we are using the Δ*recA* derivative of AAEC072 (strain AAEC078) to obtain further characterization of the roles of FimB and FimE in the *fim*-specific DNA inversion event. As expected, the new Δ*fim* strains do not support the site-specific DNA inversion when transformed with the invertible element alone (data not shown).

Experimental procedures

Bacterial strains, plasmids and growth media

All strains are derivatives of *E. coli* K12. HB101 was from our own or P. Klemm's laboratory. MG1655 (CGSC6300) and P678-54 (CGSC4928) were from B. Bachmann. VL584 was from our own laboratory stocks. Strains MM294 and YMC9 were purchased from ATCC. Plasmid pIB310 was constructed from cloned *fim* DNA from MG1655 (our unpublished data) and a derivative of the temperature-sensitive vector, pMAK705 (Hamilton *et al.*, 1989). The different regions of *fim* homology are separated by a 10bp *Bam*HI linker (Pharmacia). Bacteria were cultured on LB media, supplemented with antibacterial agents as necessary (Maniatis *et al.*, 1982) at 37°C unless otherwise stated. The *fim* deletion mutants were isolated on sucrose agar (LB agar with 6% sucrose, but lacking sodium chloride) at 30°C.

Recombinant DNA techniques

All methods have been described by Maniatis *et al.* (1982). Restriction endonucleases and T4 ligase were obtained from BRL.

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