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Chromosomal mapping of genes encoding mannose-sensitive (type I) and mannose-resistant F8 (P) fimbriae of *Escherichia coli* O18:K5:H5

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1. SUMMARY

DNA hybridization experiments demonstrated that the gene clusters encoding the F8 fimbriae (*fei*) as well as the type I fimbriae (*pil*) exist in a single copy on the chromosome of *E. coli* O18:K5 strain 2980. In conjugation experiments with appropriate donors, the chromosomal site of these gene clusters was determined. The *pil* genes were mapped close to the gene clusters *thr* and *leu* controlling the biosynthesis of threonine and leucine, respectively. The *fei* genes were found to be located close to the galactose operon (*gal*) between the position 17 and 21 of the *E. coli* chromosomal linkage map.

2. INTRODUCTION

The adherence of pathogenic *E. coli* to host cell surfaces is often the primary step in the infectious process. In many cases this cell interaction is mediated by adhesive fimbriae, which can be divided into different types (type I, P, S, M, and X fimbriae) on the basis of their receptor specificities. *E. coli* P-fimbriae associated with urinary tract infections can be subdivided into serologically distinct groups termed F7–F14 [1–3]. All of them recognize as receptor the α-D-Gal-(1–4)-β-D-Gal region of globotetraosylceramide and trihexosyl-ceramide, which are antigens of the human blood group P system [4]. *E. coli* exhibiting P fimbriae produce a mannose-resistant hemagglutination (MRH) with human erythrocytes. In contrast, type I fimbriae (F 1A) which recognizes a mannose-containing receptor are termed mannose-sensitive hemagglutination (MSH) fimbriae [5]. Genetic determinants which code for some of the P-specific fimbriae as well as for type I fimbriae have been cloned and analysed [6–12]. It has been described that in some uropathogenic *E. coli* strains copies of P-specific determinants were found to exist at multiple sites of the chromosome [13,14] whereas type I specific genes seem to be located at a fixed position on the *E. coli* chromosome [13,15, unpublished results].

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In this paper we describe experiments demonstrating that the gene cluster encoding for F8 fimbriae (F eight; *fei*) as well as for type I fimbriae (*pil*) exist in a single copy on the chromosome of *E. coli* O18:K5 strain 2980. The chromosomal site of the *fei* determinant was found to be close to the galactose operon. The *pil* determinant which has also been cloned, is located close to the *thr* and *leu* gene clusters required for threonine and leucine biosynthesis.

3. MATERIALS AND METHODS

3.1. Bacterial strains and growth media

The bacteria used are listed in Table 1. *E. coli* 2980 is a wild type strain of serotype O18:K5:H5:F8. The *E. coli* donor 20915 was obtained by introducing the F'*lac*::Tn10 plasmid from the auxotrophic *E. coli* K-12 strain NK5549 into the prototrophic *E. coli* 2980 as described previously [16]. This strain was used as a donor in crosses with the streptomycin-resistant *E. coli* K-12 recipients 2442 and P678. *E. coli* P678 is a type-I-fimbriae deficient E. coli K-12 derivative and was used here in experiments to map the *pil* genes of *E. coli* 20915. For cloning experiments, *E. coli* HB 101 was used. The origin and transfer direction of the donor *E. coli* K-12 HfrH is depicted in Fig. 1. Bacteria were grown in L-broth or on L-agar plates.

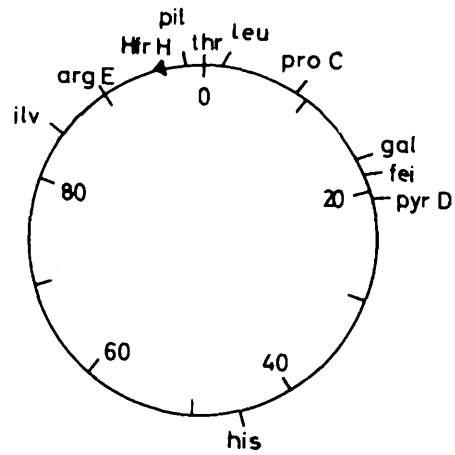

Fig. 1. Linkage map of *E. coli* with the map position of relevant markers, the *pil* and *fei* gene clusters of strain 2980 and the transfer orientation of HfrH.

3.2. Mating conditions

Intraspecific crosses with *E. coli* K-12 donor HfrH were performed in liquid medium as described earlier [18]. For crosses of *E. coli* 20915 with K-12 recipients, the mating conditions were those as described by Hull et al. [9]. Briefly, 5 ml amounts of the recipient and the donor were filtered through a 0.45 μm pore membrane filter. The filter was transferred to a L-agar plate and

Table 1

Strains used

E. coli strain	Relevant properties	MRH [a]	MSH [b]	Reference
2980	O18:K5:H5:F1:F8	+ + +	+ + +	[11]
20915	as 2980 with F'*lac*::Tn10	+ + +	+ + +	[16]
HfrH	K-12 donor, *thi*	−	+ + +	[17]
2442	K-12, F⁻ *his*, *arg*E, *pro*C, *gal*K *pyr*D, *rps*L (as AB1133 with *pyr*D)	−	+ + +	[17]
2442-1	*pyr*D⁺ hybrid from 20915 × 2442	+ + +	+ + +	this study
2442-2	*pyr*D⁺ hybrid from 20915 × 2442	+ + +	+ + +	this study
P678	K-12, F⁻ *thr*, *leu*, *gal*K, *rps*L	−	−	[9]
P678-1	*thr*⁺ *leu*⁺ hybrid from 20915 × P678	−	+ + +	this study
P678-2	*thr*⁺ *leu*⁺ hybrid from 20915 × P678	−	+ + +	this study
HB101/pANN920	cosmid clone harboring *fei* genes of 2980	+ + +	−	[11]
HB101/pMSH2980	cosmid clone harboring *pil* genes of 2980	−	+ + +	this study

[a] MRH, agglutination of human erythrocytes (P⁺) in the presence of D-mannose.

[b] MSH, agglutination of guinea pig erythrocytes or *Saccharomyces* cells in the absence of D-mannose.

incubated for 4 h at 37 °C. The filter given into 5 ml saline was blended vigorously in a Vortex mixer for 15 min. The bacteria were sedimented, washed and plated on selective medium with 100 μg streptomycin per ml for counter-selection of the donor strain. Selections of hybrids were achieved on appropriately supplemented minimal agar as described earlier [18].

3.3. Isolation of cosmid clones

A cosmid gene library of strain 2980 was constructed as described [11]. Mannose-resistant and mannose-sensitive hemagglutinating clones were selected and characterized (see Table 1).

3.4. Characterization of fimbriated strains

Wild type bacteria, *E. coli* hybrids and clones (cosmid carrying strains) which express P fimbriae agglutinated erythrocytes having P blood group antigens in the presence of D-mannose. Type-1 fimbriae were detected by agglutination of human and guinea pig erythrocytes and *Saccharomyces* cells in a mannose-sensitive manner.

3.5. Isolation of fimbriae and polyacrylamide gel electrophoresis (PAGE)

Fimbriae were isolated from bacteria as described earlier [19]. P fimbriae were disintegrated by boiling for 5 min in 10 mM Tris-HCl (pH 7.8) containing (in ml) 4% sodium dodecyl sulfate (SDS), 0.01 ml mercaptoethanol, 0.2 ml glycerol, and 0.002% bromphenol blue [19]. This procedure was not applicable to type-I fimbriae which were dissociated by incubation in saturated guanidinium hydrochloride at 37 °C as described by Eshdat et al. [20]. The pretreated samples were run on slab gels as described previously [19].

3.6. Nick translation, Southern hybridization and autoradiography

The DNAs were treated with suitable restriction enzymes and the resulting fragments were separated by agarose gel electrophoresis using 0.7–1% gels [11]. The transfer of DNA fragments from agarose gels to nitrocellulose filters, washing and autoradiography were performed as described by Southern [21]. Stringent conditions were used for the washing procedures. DNA fragments were labeled by nick-translation with a mixture of all 4 α-^{32}P-labeled dNTPs and purified by ethanol precipitation. As DNA probes a 6.0 Kb *Kpnl–Hin*dIII fragment derived from plasmid pANN921 (specific for the P determinants; see [11]) and a 4.2 Kb *Bam*HI–*Cla*I fragment of plasmid pPKL38 (specific for the type-I determinants; see [22]) were used.

4. RESULTS

4.1. Genetic linkage of fimbrial determinants

From the conjugational cross of *E. coli* donor strain 20915 with *E. coli* K-12 recipient 2442 hybrids were selected for inheritance of genes required for pyrimidine, arginine, proline and histidine biosynthesis and galactose fermentation. The resulting hybrids were tested for co-inheritance of the donor MRH$^+$ phenotype which was taken as evidence for the expression of F8 fimbriae. Although the yield of hybrids was very poor it soon came to light that the MRH$^+$ phenotype must be associated with *gal* and *pyr*D genes of the donor (see Table 2). Moreover, two of the three *pro*C$^+$ hybrids exhibiting the MRH$^+$ phenotype had also obtained the intact *gal* operon of the donor indicating the rather close linkage between the MRH$^+$ phenotype and *gal*.

In order to find out whether or not the *pil* gene cluster responsible for the production of type-I (MSH) fimbriae is located next to the genes required for leucine and threonine biosynthesis suitable conjugal crosses were carried out. The type-I-fimbriae deficient *E. coli* K-12 F$^-$ strain P 678 was used as a recipient in crosses with the donor

Table 2

Inheritance of the donor MRH$^+$ phenotype in a cross of *E. coli* 20915 (MRH$^+$) and *E. coli* 2442 (MRH$^-$)

Selected marker	Total no. of hybrids	No. of hybrids with MRH$^+$ phenotype
his$^+$	26	0
*arg*E$^+$	14	0
*pro*C$^+$	7	3
*gal*K$^+$	9	8
*pyr*D$^+$	6	3

Table 3

Inheritance of MRH^+ and MSH^+ phenotype in a cross of *E. coli* 20915 (MRH^+, MSH^+) with *E. coli* P678 (MRH^-, MSH^-)

Selected marker	Total no. of hybrids	No. of hybrids with the phenotype		
		MRH^+ MSH^-	MSH^+ MHR^-	MRH^+ MSH^+
thr$^+$ *leu*$^+$	12	0	10	0
gal K^+	10	6	0	2

E. coli 20915. The outcome of this cross (Table 3) showed that the MSH^+ phenotype of *E. coli* 20915 is transferred at high frequency with the *thr–leu* region of the donor chromosome. When selections from this cross were made for the intact donor *gal* operon (*gal*$^+$) 8 out of 10 hybrids had obtained the MRH^+ phenotype. Interestingly, the two *gal*$^+$ hybrids which in addition exhibited the MSH^+ phenotype had also obtained the unselected donor genes for the biosynthesis of threonine and leucine. This result suggests that the *pil* gene cluster is closely linked to the *thr–leu* region and is widely separated from the *fei* determinant in *E. coli* 2980.

4.2. Characterization of fimbriated hybrid strains

The presence of F8 fimbriae in the hybrids was further confirmed by the isolation of fimbriae and by subsequent SDS-PAGE. After purification and dissociation of fimbriae with SDS, the 21 kD protein subunits characteristic for the F8 fimbriae of *E. coli* 2980 (see Fig. 2) were obtained also from hybrids expressing the donor MRH^+ phenotype. As an additional control, a cosmid clone harboring the *fei* determinants of *E. coli* 2980 [11] and producing F8 fimbriae was used (Fig. 2). The recipient K-12 strain 2442 served as a fimbriae-negative control.

Type I fimbriae were isolated from MSH^+ hybrids. The recipient K-12 strain P678 was used as a fimbriae-negative control. After dissociation with saturated guanidine hydrochloride the fimbrial preparations of MSH^+ hybrids showed fimbrial subunits in SDS-PAGE which were identical with those obtained from *E. coli* 2980 and from the K-12 cosmid clone (HB101/pMSH2980) harboring the *pil* genes of *E. coli* 2980 (Fig. 2).

4.3. Southern hybridizations of hybrid strains

In order to confirm the identity of the fimbrial-specific determinants of *E. coli* 2980 with those of the hybrids and the cosmid clones DNA-DNA hybridizations were carried out. DNAs of the wild type *E. coli* 2980, the hybrids and the cosmid clones were digested with *Pst*I and hybridized with a P-specific DNA probe [11]. As shown in Fig. 3 (lanes A-C, F) identical fragments were obtained from the different DNAs isolated from the wild-type strain 2980, the exconjugants 2442-1 and 2442-2 and from the cosmid clone HB101/pANN920. After cleavage of the DNAs from the wild-type strain, two exconjugants and a cosmid clone with *Pst*I and subsequent hybridization with a type-I-specific probe fragments of

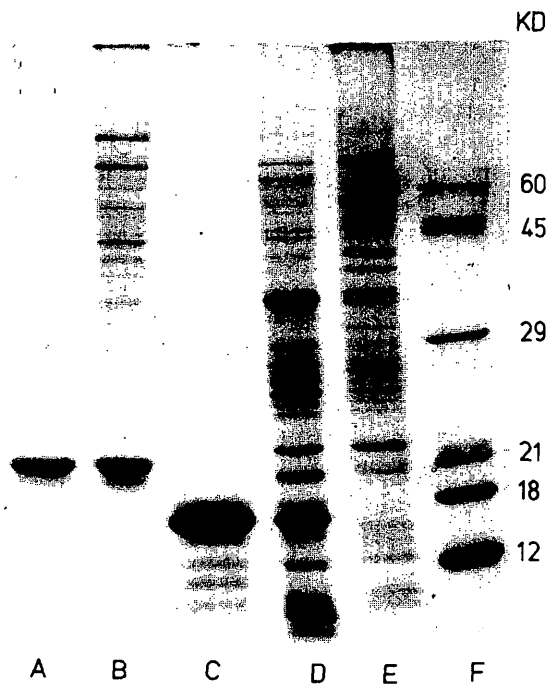

Fig. 2. SDS-PAGE of type I and F8 fimbrial subunits from *E. coli* 2980 and *E. coli* K-12 clones. Approx. 5 μg fimbriae were mixed with 20 μg buffer. Lanes: (A) 2980 (*fei*$^+$, wild-type); (B) HB101/pANN920 (*fei*$^+$, cosmid clone); (C) 2980 (*pil*$^+$, wild-type); (D) HB101/pMSH2980 (*pil*$^+$, cosmid clone); (E) HB101; (F) M_r-marker proteins.

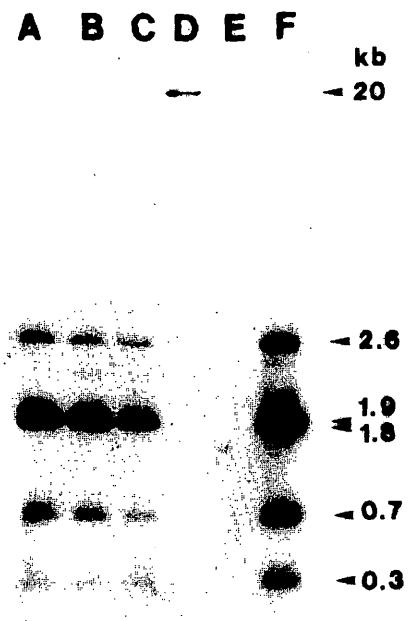

Fig. 3. Hybridization pattern of PstI- (Lanes A, B, C, E, F) and *Sal*I (Lane D) DNAs from strains 2980 (*fei*$^+$, wild-type, Lanes A and D); 2442-1 (*fei*$^+$, exconjugate, Lane B); 2442-2 (*fei*$^+$, exconjugate, Lane C); 2442 (*fei*$^-$, K-12 recipient, Lane E); HB101/pANN920 (*fei*$^+$, cosmid clone, Lane F). As hybridization probe a nick-translated α-^{32}P = labeled 6.0 kb *Kpn*I-*Hin*dIII fragment of pANN921, specific for P-fimbrial determinants [11] was used.

comparable sizes were also observed (Fig. 4). These results argue for an identity of the *fei* determinants of the wild-type and the exconjugants and the same is true for the *pil* gene clusters of the different strains.

In addition, the DNA of *E. coli* 2980 was digested with *Sal*I which does not cleave the *fei* and the *pil* determinants [11,22]. After hybridization with the P-and type-I-specific probes only one major band was seen which strongly suggests that only one P and one type I determinant are present on the chromosome of *E. coli* 2980 (see Figs. 3 and 4, Lanes D).

4.4. Determination of the precise map position of the fei *genes*

In order to find out whether the chromosomal site of the *fei* genes is within the *pyr*D–*gal* or

Table 4

Inheritance of the donor MRH$^-$ phenotype among different recombinant classes of a cross between *E. coli* HfrH (MRH$^-$) and 2442-1 (MRH$^+$)

Recombinant class	Total no. of hybrids	No. of hybrids with phenotype		Transfer frequency of MRH$^-$ phenotype (%)
		MRH$^+$	MRH$^-$	
proC$^+$ *gal*$^+$	139	58	81	58
proC$^+$ *gal*$^-$	75	75	0	0
proC$^-$ *gal*$^+$	13	7	6	46

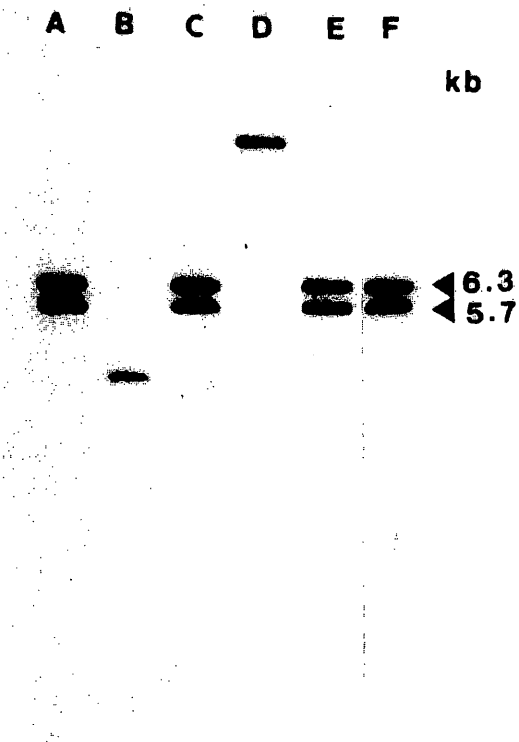

Fig. 4. Hybridization pattern of *Pst*I- (Lanes A, B, C, E, F) and *Sal*I- (Lane D) DNAs from strains 2980 (*pil*$^+$, wild-type, Lanes A and D); P678 (K-12) recipient, *pil*$^-$, Lane B); P678-1 (*pil*$^+$, *thr*$^+$, *leu*$^+$, exconjugate, Lane C); P678-2 (*pil*$^+$, *thr*$^+$, *leu*$^+$-, exconjugate, Lane E); HB101/pMSH2980 (*pil*$^+$, cosmid clone, Lane F). As hybridization probe a nick-translated α-^{32}P-labeled 4.2 kb *Bam*HI–*Cla*I probe of pPKL38, specific for Type I determinants [22] was used.

within the *gal–pro*C region (see Fig. 1) further crosses were made with the *E. coli* donor HfrH and the *E. coli* K-12 F^- strain 2442-1 as recipient. *E. coli* 2442-1 is a *pyr*D^+ *fei*$^+$ hybrid from the crossing between *E. coli* 20915 and 2442 and exhibits the MRH^+ phenotype. This hybrid is still auxotrophic for proline (*pro*C^-) and does not ferment galactose (gal^-). Hybrids were selected for the inheritance of the intact donor genes *pro*C^+ and/or *gal*$^+$ and were tested for co-inheritance of the MRH^- phenotype of the HfrH donor. The results, as summarized in Table 4, provide some information on the order of the gene loci in question. The order *fei–gal–pro*C seems to be the most probable one because no quadruple cross-over would be required among the 227 hybrids tested here. Considered together with the results in Table 2 we suggest that the gene order is *pyr*D–*fei*–*gal–pro*C.

5. DISCUSSION

Several attempts have been made to find out whether the gene clusters coding for fimbrial adhesions are located on the chromosome or on plasmids. It was shown that the great majority, if not all of adhesin determinants of extra-intestinal *E. coli,* are chromosomally encoded. In addition, it is generally accepted that *E. coli* cells of one strain may carry more than one adhesin determinant on their chromosomes [8,12]. Using conjugative bacterial transfer experiments it was demonstrated that gene clusters coding for type I fimbriae (*pil* or *fim* determinants) are located in the vicinity of the *thr* and *leu* gene loci at map position 98 [15]. This was shown for *E. coli* K-12 wild type strains of *E. coli* 04 and 083 (13, 15 unpublished results). In experiments described in this paper it was found that also in the *E.coli* O18:K5 strain 2980 the *pil* gene cluster is located at this particular chromosomal site.

The genetic determinants which code for P and P-related fimbriae (serotypes $F7_1$, $F7_2$, F13) are mapped in regions close to the *ser*A gene (map position 57–63), at position 85–90 close to the *ilv* gene cluster and at position 95 on the chromosome of 04 and 06 wild type strains [13,14]. As shown in this paper the genetic determinants of F8 fimbriae were mapped at a position between 17–20 on the chromosome of strain 2980 of *E. coli* O18:K5. Therefore, it seems that the gene clusters coding for type I fimbriae are located at fixed positions on the chromosomes of different strains. In contrast, P-fimbrial determinants may be located in different regions on the chromosomes of extra-intestinal *E. coli* isolates.

Very often the genetic determinants of P or P-related fimbriae are linked to gene clusters coding for hemolysin (*hly*), another pathogenicity factor of *E. coli* [23]. Such a linkage has been described for different *E. coli* serotypes, including 04, 06 and 018 strains [14,24–26; Hacker et al. in preparation]. In *E. coli* 2980 no such linkage could be found to exist since none of the MRH^+ hybrids were able to produce hemolysin. Recently, however, in our laboratory the *hly* and a P-specific determinant of another O18:K5 strain (B2020) could be cloned together into the cosmid vector pHC79 [unpublished results], thereby indicating a very close linkage of these determinants in that particular isolate. This clearly indicates that even in bacteria exhibiting the same O:K serotype the arrangement on the chromosome of the genetic determinants of these two pathogenicity factors may be quite different.

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