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Mutations Affecting the Citrate-Dependent Iron Uptake System in *Escherichia coli*

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Isolation of six strains of *Escherichia coli* K-12 carrying mutations affecting the citrate-dependent iron uptake system is described. Genetic analysis of these mutants showed that the mutations affecting the citrate system are clustered together at about min 6 on the *E. coli* chromosome.

Escherichia coli K-12 is known to possess several alternative systems for transporting iron. In the absence of suitable iron-chelating ligands in the medium, cells of *E. coli* excrete enterochelin and transport iron by the enterochelin system (see reference 13). *E. coli* can also utilize several other iron-chelating ligands produced by other organisms to promote iron transport (6, 9, 12). In the presence of citrate, *E. coli* can use a citrate-dependent iron transport system (5, 6, 10). In the present work, we describe the isolation and genetic analysis of mutants affected in the citrate-dependent iron transport system.

Strains with mutations affecting the enterochelin system are unable to grow under iron-deficient conditions, but their growth can be stimulated by the addition of citrate to the growth medium (5, 10). Under these conditions, they use the citrate-dependent system for iron transport. By making use of a strain affected in the common pathway of aromatic biosynthesis, the production of enterochelin can be readily controlled and the strain made dependent on the citrate system for iron transport. Six mutants affected in the citrate system were isolated after mutagenesis of strain AN92 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Strain AN92 was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine under the conditions described by Adelberg et al. (1). The cells were then allowed to grow for about two generations in nutrient broth, washed twice with basal mineral salts medium (14, 17), and plated onto a glucose-mineral salts agar medium containing 1 mM sodium citrate, no added iron, and no precursor of enterochelin (10, 18). The plates were incubated for 4 days at 37°C, and all colonies were marked. An agar overlay containing 0.5 mM shikimate was then added, and the plates were incubated for another 4 days. All colonies appearing after enrichment were tested for their ability to grow on low-iron media containing 1 mM citrate or 0.1 mM 2,3-dihydroxybenzoate. Six mutants that were unable to grow in the presence of citrate but that grew normally with 2,3-dihydroxybenzoate were isolated and retained for further study.

The genes affected in the mutants were designated *fec*. Strain AN300, carrying the allele *fec-403*, was used in conjugation experiments with several Hfr strains (see Table 1 for details of the strains used). The matings were performed as described previously (15), and *fec*$^+$ recombinants were selected by plating the mating mixture onto succinate-minimal medium supplemented with 10 mM citrate in the absence of a precursor of enterochelin. Strain AN300 carries an *aroB* allele that prevents the formation of enterochelin precursors. The *fec*$^+$ gene was transferred as an early marker into strain AN300 by both Hfr strains AB259 and AB261. This indicated that the *fec* gene lies between the origins of these two Hfr strains, i.e., between min 96 and 7 (Fig. 1).

Attempts were made to cotransduce the *fec-403* allele with several genes (*proA, proB* and *argF*) located in this region of the chromosome; cotransduction with the *argF* gene was shown (Table 2). Difficulty was experienced in obtaining *arg*$^+$ transductants, and a comparison with the number of *ilv*$^+$ transductants obtained in parallel experiments showed that the number of *arg*$^+$ transductants was abnormally low (Table 2). Transduction experiments with the *argF* marker are complicated by the presence of a second gene, *argI* at min 95 (8), that codes for ornithine transcarbamylase, as does *argF*. Therefore, cotransduction of *fec* and *argF* was tested by using a P1 phage lysate of strain AN300 (*fec-403*) as the donor and strain AN365 (*argF argI*) as the recipient and selecting for *arg*$^+$. Any observed transfer of the *fec* marker

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TABLE 1. *Strains of E. coli K-12 used*

Strain	Sex	Relevant genetic loci[a]	Source or other information
AN92	F⁻	*proA argE pheA tyrA trp-401 aroB rpsL*	Parent strain of *fec* mutants
AN368	F⁻	*pabA his-4 argI ilv-7 aroE rpsL spc^r fec-403*	*argF*⁺ transductants derived with AN300 as donor and AN365 as recipient
AN369	F⁻	*pabA his-4 argI ilv-7 aroE rpsL spc^r*	
AB259	Hfr	*thi*	Hfr Hayes
AB261	Hfr	*metB*	
AB2332	Hfr	*met thi*	Hfr Cavalli

[a] Genetic nomenclature is that used by Bachmann et al. (2).

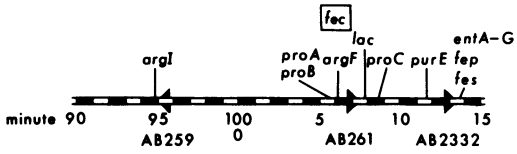

FIG. 1. *Segment of the genetic map of E. coli showing the origins of the Hfr strains used and the approximate positions of relevant genes (2).*

would indicate linkage to the *argF* gene, as the conjugation experiments eliminate the possibility of any close linkage of the *fec* gene to the *argI* gene. One of the *argF*⁺ *fec* transductants was tested in iron uptake experiments (Fig. 2) and was found to be deficient in citrate-mediated iron uptake, confirming that the *fec-403* allele had been transferred by cotransduction.

Transduction experiments with the remaining five *fec* strains showed cotransduction of *argF* and *fec* genes in only two strains (AN302 and AN306) (Table 2). In all cases, the number of *arg*⁺ transductants was again low. Further conjugation experiments with strains AN298, AN299, and AN303 and the Hfr strains AB259 and AB261 showed that the *fec* alleles that were not cotransducible with *argF* were also in the same region of the chromosome as the *fec-403* allele. Further interrupted-mating experiments with the Hfr strain AB2332 and the female strain AN299 (*fec-402*) showed that the *fec-402* allele was close to the *proA* gene. However, transduction experiments failed to show cotransduction between the *proA* and *fec* genes.

The data presented above indicate that the six mutations affecting the citrate-dependent iron transport system are located at about min 6 on the *E. coli* chromosome in the vicinity of the *argF* gene. It is important to note that the *fec* gene(s) is well separated from the *ent* gene cluster at min 13, which codes for proteins concerned with the enterochelin system of iron transport, and from the *tonA* gene (min 3), which is involved in the ferrichrome system (9). Iron uptake studies showed that the *fec* mutants are unaffected in both ferrichrome- and enterochelin-mediated iron uptake (P. Wookey, unpublished data). The properties of the *fec* mutants and the position of the *fec* gene(s) indicate

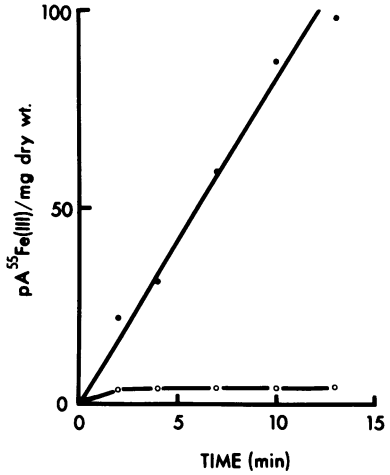

FIG. 2. *Citrate-mediated iron uptake in fec⁺ and fec strains. Strains were grown overnight in the presence of 1 mM citrate and 5 μM 2,3-dihydroxybenzoate. The cells were then starved, and uptake measurements of ⁵⁵Fe(III) were made as described elsewhere (6, 10). The uptake medium contained 1 μM ⁵⁵Fe(III), 1 mM citrate, and 100 μM nitrilotriacetate. Symbols: ○, AN368 (fec-403); ●, AN369 (fec⁺).*

TABLE 2. *Transduction data for fec strains*[a]

Donor strain	Recipient strain	No. of *arg*⁺ transductants	No. of *ilv*⁺ transductants	No. of *arg*⁺ *fec* transductants
AN300 (*fec-403*)	AN365 (*argF ilv*)	70	620	8 (11%)
AN302 (*fec-405*)	AN365 (*argF ilv*)	138	660	11 (8%)
AN306 (*fec-409*)	AN365 (*argF ilv*)	34	194	1 (3%)

[a] Transductions with P1*kc* were carried out as described by Pittard (11).

that *fec* mutants are distinct from any of the other mutants affected in iron transport described previously (2–5, 7, 9, 12). Although accurate mapping of the *fec* alleles by cotransduction has been hampered by an apparent lack of homology between the strains used, three of the *fec* alleles were shown to be cotransducible with *argF*. Other workers have also obtained conflicting data for markers in the *proA-lac* region, and it has been suggested that different *E. coli* K-12 strains may not be homologous for this region of the chromosome (16).

One of the current problems in the study of the iron transport systems of *E. coli* is the elucidation of the role of both outer membrane and inner membrane proteins in the active transport of the various iron complexes (3, 4, 7). The various *fec* alleles described in the present work should prove useful in characterizing the role of outer and inner membrane components in the citrate-dependent system.

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