JOURNAL OF BACTERIOLOGY, Nov. 1976, p. 604-608

Vol. 128, No. 2
Printed in U.S.A.

tolF Locus in *Escherichia coli*: Chromosomal Location and Relationship to Loci *cmlB* and *tolD*

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Received for publication 3 May 1976

The tentative map position on the *Escherichia coli* chromosome of the *tolF* locus, determining tolerance to colicins A, E2, E3, K, and L, has been confirmed by three-point transduction. It lies between the *aroA* and *pyrD* loci at about 21 min on the linkage map of Bachmann et al. (1976). The *cmlB* locus, determining increased resistance to the antibiotics chloramphenicol and tetracycline, also lies in this region (Reeve, 1966). Phenotypic and genetic comparison of isogenic strains that carry a mutation in either the *tolF* or *cmlB* locus makes it likely that these loci are closely related or identical. The *tolD* locus determining tolerance to colicins E2 and E3 as well as increased resistance to antibiotics has been reported to be located close to the *aroA* locus as a result of conjugation experiments (Eriksson-Grennberg et al., 1965). However, *tolD* did not cotransduce with any of several loci in this region, indicating that the mutation is not located within the region of the genetic map corresponding to approximately 19 to 22.5 min.

Serratia marcescens strain JF246 produces a bacteriocin (8) that is apparently closely related to colicin L produced by *Escherichia coli* strain 398. Antibody prepared against pure bacteriocin JF246 inactivated colicin L-398. In addition, mutants selected as tolerant to bacteriocin JF246 (10) were also tolerant to colicin L-398 and vice versa. Thus, following the suggestion of Fredericq (11) and P. Reeves (personal communication), I will refer to this material as colicin L-JF246.

tolF mutants were selected as tolerant to colicin L-JF246 (10), whereas *cmlB* mutants were selected as resistant to chloramphenicol (14). *tolF* mutants were shown to have altered sensitivities to a variety of colicins, dyes, and detergents (10). *cmlB* mutants were shown to have increased resistance to chloramphenicol and tetracycline (14). This paper demonstrates that isogenic strains carrying *tolF* or *cmlB* have identical phenotypic responses to a variety of colicins, bacteriophages, dyes, detergents, and antibiotics. Genetic mapping and complementation tests confirm this identity.

Until the nature of the *tolF* and *cmlB* gene product is clarified, I propose that this locus be called *tolF*. Tolerance to colicins is a more striking phenotypic effect than increased resistance to antibiotics. The colicin tolerance in some cases extends over a 4,000-fold concentration range, whereas the increase in resistance to antibiotics extends over a two- to threefold range.

A *tolF* strain was compared with a *tolD* strain for two reasons. First, the *tolD* strain was selected as a spontaneous mutant resistant to an antibiotic (ampicillin) (7) and was later characterized as tolerant to colicins E2 and E3 and somewhat resistant to chloramphenicol (2). Second, the *tolD* mutation was located by conjugation experiments on the genetic map near the *tolF* locus (2).

I have demonstrated that the *tolF* can be cotransduced with *pdxC*, *aroA*, *pyrD*, and *fabA*, whereas *tolD* does not cotransduce with any of these loci. Thus the *tolF* and *tolD* loci are distinct.

MATERIALS AND METHODS

Microorganisms and media. *E. coli* K-12 strains used for these studies are described in Table 1. The medium has been previously described (10). Normally, cells were grown in L broth containing 2.5 mM $CaCl_2$ or minimal medium supplemented with 0.2% glucose, streptomycin sulfate (100 μg/ml), and appropriate growth supplements. These media were solidified with 1.5% agar or 0.7% agar (soft agar). Bacteriophage P1*vir* was obtained from John Cronan, and phage f2 was obtained from Peter Model.

Genetic procedures and strain construction. Before a conjugation experiment, the mating ability of the F′ donor culture was confirmed first by demonstrating sensitivity of the culture to phage f2 and second by demonstrating the ability of the culture to donate appropriate genetic markers to a *recA* recipient. Growth of parental strains was generally inhibited by streptomycin or by the omission of re-

TABLE 1. *Escherichia coli K-12 strains*

Strain	Genotype[a]	Reference or source
AB2856	*aroA357 ilv-7 arg-3 thi-1 pro-2 his-4*	(14)
AT3143	*pdxC3 pyrF ilv-277 metB65 his-53 purE41 proC24 cyc-1 xyl-14 lacY29 str-77 tsx-63*	A. L. Taylor
CY99	*pyrD34 thr-1 his-1 trp-1 galG xyl-7 mtl-2 recA1 str-118 F'106 pyrD*$^+$	J. Cronan
G11e1	*ilv metB tolD* (HfrC)	(2)
JF404-4a	*thyA tolFf* (HfrH)	(10)
JF404-11a	*thyA tolF11* (HfrH)	(10)
JF556	KL185, *aroA357 thyA*[b]	–[c]
JF557	JF556, *aroA*$^+$ *tolF4*[b]	–
JF558	JF556, *aroA*$^+$ *cmlB*[b]	–
JF560	RE110 *recA*[b]	–
JF561	JF557, *recA tolF4*[b]	–
JF562	JF558, *recA cmlB*[b]	–
JF568	AT3143, *pyrF*$^+$ *pdx*$^+$ *aroA357*[b]	–
KL16-99	*recA thi ser-11 drm-3 relA*	B. Low
KL185	*thi-1 pyrD34 his-68 trp-45 mtl-2 xyl-17, galK35 str-118* λ^r, λ^-	B. Bachmann
RE107	*proA trp his lacY ton cmlB strA*	(14)
RE110	*pyrD galE*	(16)
UC1098	*fabA2 strA*	(4)

[a] Genetic nomenclature of Bachmann et al. is used (1).

[b] Unless otherwise specified, all other markers are the same as in the indicated parental strain.

[c] Preparation of strain described in this paper.

quired supplements in the plating medium to allow development and identification of merodiploid exconjugants. Growth of phage P1*vir* and transduction procedures were as described by Signer (19). Direct selection of *tolF* or *cmlB* transductants was not successful, presumably due to a long lag before expression of the phenotype. This may reflect a requirement for complete removal of a component of the wild-type cell envelope before a transductant is phenotypically tolerant to colicin.

Construction of isogenic strains that carried either *tolF* or *cmlB* mutations was accomplished by first introducing *aroA357* into strain KL185. Phage P1*vir*, grown on strain JF568, was used to infect strain KL185, and after removal of unabsorbed phage, the *aroA357* transductants were enriched, isolated, and purified by using the penicillin enrichment technique described by Rossi and Berg (17). A *thyA* mutant was isolated from this *aroA pyrD* strain by using trimethoprim (20), and the resulting strain was labeled JF556. Next, phage P1*vir* lysates prepared on either strain JF404-4a or strain RE107 were used to introduce *aroA*$^+$ *tolF* or *aroA*$^+$ *cmlB*, respectively, into strain JF556 by transduction. These transductants were labeled JF557 and JF558 (Table 1). The *recA* derivative of strains JF557 and JF558 was prepared by conjugation with strain KL16-99 previously described (10), except that *recA* recombinants were picked, purified, and characterized from among a population of *thyA*$^+$ recombinants. The *recA* strains were labeled JF561 and JF562. Strain JF560 was prepared from a *thyA* derivative of strain RE110 by the same procedure. Sensitivity to colicins was determined by the plate assay previously described (10). Sensitivity to detergents, dyes, and ethylenediaminetetraacetic acid was determined as described by Davies and Reeves (5). Sensitivity to antibiotics was determined by using Sensi-Discs obtained from BBL, Cockeysville, Md. Up to 12 discs were placed on a single LC agar plate that had been overlaid with 2.5 ml of LC soft agar containing about 10^7 cells. After overnight incubation, sensitivity was scored by measuring a distinct zone of inhibition of growth of the test strain, whereas resistance was expressed as no detectable inhibition or a markedly decreased (at least 3 mm in diameter) size of zone of inhibition.

RESULTS

Position of *tolF* and *cmlB* loci on the genetic map. Two independently isolated spontaneous *tolF* mutants in strain HfrH(JF404) were selected for transduction studies. For most studies, however, only the allele in strain JF404-4a was used. Table 2 describes the linkage of *tolF* and *cmlB* to several loci located between 20 and 21.5 min on the genetic map of Bachmann et al. (1). Clearly, *tolF* and *cmlB* share similar linkage relationships with *pyrD* and *pdxC*. (Compare Table 2, crosses 1, 2, 3, 5, and 6 with crosses 7 and 8).

The three-factor transduction experiments summarized in Table 3 position both the *tolF* and *cmlB* loci between *aroA* and *pyrD*. The data in Table 4 report my attempts to separate *tolF* and *cmlB* loci by transduction. The mutations in these strains are closely linked but not identical. Two *tolF*$^+$ recombinants were observed among 1,750 *pyrD*$^+$ transductants. *pyrD*$^+$ recombinants were also selected after conjugation between HfrH strain JF404-4a (*pyrD*$^+$ *tolF*) and strain JF558 (*pyrD cmlB*). All of 800 *pyrD*$^+$ recombinants tested were resistant to colicin A (*tolF* or *cmlB*). The low frequency of recombination between the *tolF* and *cmlB* strains indicates that the two mutations are located close to one another on the chromosome and may affect the same gene. The position of *tolF* on the genetic map is presented in Fig. 1, which summarizes the results in Tables 2 and 3.

TABLE 2. *Cotransduction of tolF and cmlB loci with loci close to pyrD locus*

Cross no.	Bacterial strains and relevant markers		Selected marker	Transductants with unselected donor marker/total transductants tested	Cotransduction frequency (%)
	Donor	Recipient			
tolF locus					
1	AT3143 (*pdxC*)	JF557 (*pyrD tolF4*)	*pyrD*$^+$	151/250 *tolF*$^{+a}$	60
				23/250 *pdx*	9.2
2	JF557 (*pyrD tolF4*)	AT3143 (*pdxC*)	*pdxC*$^+$	134/250 *tolF*	54
				17/250 *pyrD*	6.8
3	UC1098 (*fabA*)	JF557 (*pyrD tolF4*)	*pyrD*$^+$	116/200 *tolF*$^+$	58
				120/200 *fabA*	60
4	JF557 (*pyrD tolF4*)	UF1098 (*fabA*)	*fabA*$^+$	91/250 *tolF*	36
				125/250 *pyrD*	50
5	JF404-4a (*tolF4*)	JF544 (*pyrD*)	*pyrD*$^+$	53/100 *tolF*	53
6	JF404-11a (*tolF11*)	JF544 (*pyrD*)	*pyrD*$^+$	61/100 *tolF*	61
cmlB locus					
7	RE107 (*cmlB*)	KL185 (*pyrD*)	*pyrD*$^+$	145/250 *cmlB*	58
8	RE107 (*cmlB*)	AT3143 (*pdxC*)	*pdxC*$^+$	141/250 *cmlB*	56
9	RE107 (*cmlB*)	AB2856 (*aroA*)	*aroA*$^+$	135/250 *cmlB*	54

[a] Unselected marker.

TABLE 3. *Reciprocal three-factor cotransductions of tolF or cmlB with aroA and pyrD*

Cross no.	Bacterial strains and relevant markers		Selected marker	No. selected	Distribution of unselected markers			
	Donor	Recipient						
1	AB2856 (*aroA*)	JF557 (*pyrD tolF*)	*pyrD*$^+$	250	*aroA*$^+$ *tolF*$^+$	*aroA*$^+$ *tolF*	*aroA tolF*$^+$	*aroA tolF*
					112	108	26	4
2	JF557 (*pyrD tolF*)	AB2856 (*aroA*)	*aroA*$^+$	250	*pyrD*$^+$ *tolF*$^+$	*pyrD*$^+$ *tolF*	*pyrD tolF*$^+$	*pyrD tolF*
					130	102	3	15
3	AB2856 (*aroA*)	JF562 (*pyrD cmlB*)	*pyrD*$^+$	250	*aroA*$^+$ *cmlB*$^+$	*aroA*$^+$ *cmlB*	*aroA cmlB*$^+$	*aroA cmlB*
					119	111	18	2
4	JF562 (*pyrD cmlB*)	AB2856 (*aroA*)	*aroA*$^+$	250	*pyrD*$^+$ *cmlB*$^+$	*pyrD*$^+$ *cmlB*	*pyrD cmlB*$^+$	*pyrD cmlB*
					104	125	4	17

TABLE 4. *Linkage between cmlB and tolF*

Bacterial strains		Selected marker	No. of expt	Transductants with wild type phenotype/total transductants tested
Donor	Recipient			
RE107 (*cmlB*)	JF557 (*pyrD tolF*)	*pyrD*$^+$	3	1/950
JF404-4a (*tolF4*)	JF558 (*pyrD cmlB*)	*pyrD*$^+$	2	1/800

The *tolD* locus is apparently unlinked by transduction to any of the loci in this figure, for although the *tolD* locus could be transferred by conjugation (2; unpublished observations), linkage by transduction between this locus and any of the loci studied was not observed. I found less than 0.4% cotransduction of *tolD* with *pdxC*, *aroA*, *pyrD*, or *fabA*.

Phenotype of *tolF* and *cmlB* strains. Beginning with strain JF556 (*aroA pyrD*), I constructed nearly isogenic strains by transduction. These strains differed only in that strain JF557 carried *aroA*$^+$ *tolF* from strain JF404-4a and strain JF558 carried the *aroA*$^+$ *cmlB* from strain RE107. The sensitivity of the parental strain and the two transductants to a variety of agents is shown in Table 5. The phenotypes of the *tolF* and *cmlB* strains were identical.

Complementation of *tolF* and *cmlB* by F'106. To prevent recombination between the chromosome and the episome, *recA* derivatives of strains JF557 and JF558 were prepared for use in complementation studies. Introduction of the *recA* allele slows the growth rate of these strains but does not affect their resistance to colicin A or tetracycline. After transfer of F'106 from strain CY99 to strains JF561 and JF562, several *pyrD*$^+$ merodiploids were picked and purified through two single-colony isolations on selective medium. Four merodiploid isolates from each strain were tested for the presence of F'106 by their sensitivity to the male-specific phage f2 and their ability to transfer *pyrD*$^+$ into the *pyrD recA* strain JF560. Strains that were

sensitive to the male-specific phage f2 and able to donate *pyrD*$^+$ were considered to be merodiploids. All eight strains picked and tested were merodiploids, and all eight were fully sensitive to colicin A and tetracycline. The *tolF* and *cmlB* alleles are both recessive.

***cmlB tolF* merodiploid.** Hfr strain JF404-4a was mixed with strains JF561 (*recA pyrD tolF*) and JF562 (*recA pyrD cmlB*), and *pyrD*$^+$ colonies were selected on medium containing streptomycin. Under these conditions, only a few *pyrD*$^+$ colonies appeared. Each colony probably carried an F′ episome of unknown size but containing the point of origin of strain HfrH (13). Two colonies from each mating were chosen, and the presence of the episome was confirmed by the sensitivity to phage f2 and ability to donate *pyrD*$^+$ to strain JF560 as described above. The merodiploids prepared in this way were tested for sensitivity to colicin A and tetracycline. All of four strains tested were resistant to both agents. Since the episome in each case presumably carried the *tolF* allele as well as *pyrD*$^+$, the phenotype of the merodiploids prepared from strain JF562 having the genotype *pyrD cmlB*/F′ *pyrD*$^+$ *tolF* demonstrated the lack of complementation between *cmlB* and *tolF*. This indicated that the *tolF* and *cmlB* mutations were in the same gene.

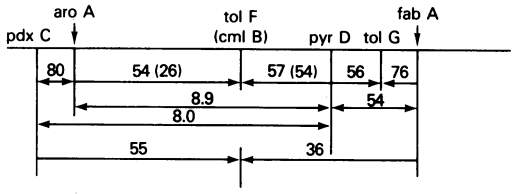

FIG. 1. *Position of tolF (cmlB) on the genetic map of Escherichia coli. The figure is an adaptation of the portion corresponding to 20 to 21.5 min on the genetic map of Bachmann et al. (1). The numbers above the arrows represent cotransduction frequencies, and the arrow heads indicate the unselected marker. Where reciprocal transductions were done (double arrow head), the cotransduction frequencies were averaged. The data are from Tables 2 and 3 except for pdxC-aroA (4) and pyrD-tolG-fabA (10). The numbers in parentheses are from Reeve and Doherty (16) for aroA-cmlB and pyrD-cmlB.*

DISCUSSION

The mutations in strain JF404-4a (*tolF*) and strain RE107 (*cmlB*) affect the same gene. This conclusion is based on similar positions on the genetic map, the low frequency of recombination between *tolF* and *cmlB*, identity of phenotype, and absence of genetic complementation. Intragenic recombination frequencies have been measured, for example within the *trpA* gene, and correlated with amino acid substitution in the gene product, the α subunit of tryptophan synthetase (12). Comparison of the frequency of recombination among mutants in the *trpA* gene with the frequency of recombination observed between *tolF* and *cmlB* suggests that the mutations in the *tolF* and *cmlB* strains are separated by only a few base pairs on the chromosome.

The relationship among *tolF*, *cmlB*, and *tolD* loci was investigated since the phenotypes have features in common. Each mutation leads to both colicin tolerance and resistance to antibiotics. Clearly, the *tolD* locus is distinct both genetically and phenotypically from the *tolF* and *cmlB* loci. The *tolD* mutation is not geneti-

TABLE 5. *Phenotypic comparison of isogenic tolF and cmlB strains*

Strain	Sensitivity to:[a]																		
	Colicin[b]													Other agents					
	A	B	D	E1	E2	E3	Ia	K	L	L-JF246	Ia	M	S1	Dyes[c]	Detergents[d]	Chloramphenicol[e]	Tetracycline	Oxytetracycline	Polymyxin B
JF556 (wild type)	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S
JF557 (*tolF*)	T	S	S	S	pT	T	S	T	T	T	S	S	S	R	R	R	R	R	R
JF558 (*cmlB*)	T	S	S	S	pT	T	S	T	T	T	S	S	S	R	R	R	R	R	R

[a] Symbols: S, sensitive; T, tolerant; pT, partially tolerant; R, resistant. Strains JF404-4a and RE107 were shown to be tolerant as previously described (10).

[b] Colicin concentrations were all 200 U/ml except for colicin Ia, which was 80 U/ml. Colicin units and strains producing colicins A, E1, E2, E3, Ia, and K have been previously described (10). Other colicin-producing strains were: AG097 (B), CA23 (D), 398 (L), 32T19F (M), P1 (31).

[c] Dyes: 400 μg of eosine yellow/ml; 100 μg of acridine orange/ml; 100 μg of methylene blue/ml.

[d] Detergents: 1% sodium dodecyl sulfate; 1% sodium deoxycholate; 0.1% Triton X-100.

[e] Antibiotic sensitivity determined by the disk sensitivity test.

cally linked by transduction to either *aroA* or *pyrD* and produces a distinct pattern of tolerance to various colicins (2). Still, the question remains as to why mutations in separate loci should have similar pleiotropic effects. The pleiotropic effects of these mutations, colicin tolerance and increased resistance to antibiotics, may be explained by viewing the outer membrane of *E. coli* both as the initial permeability barrier of the cell and the primary site of interaction of colicins with the cells. Alterations in the composition or arrangement of outer membrane components (primarily phospholipids, protein, and lipopolysaccharide) could produce pleiotropic effects such as have been described here for *tolF*, *cmlB*, and *tolD* strains. For example, increased resistance to antibiotics may be due to decreased permeability of the outer membrane to these agents. Presumably the alteration in the membrane that results in decreased permeability may also change the sensitivity of the cell to colicins.

Although I do not wish to suggest that all colicin-tolerant mutants have an altered outer membrane, at least some do. *tolG* mutants lack a major outer membrane protein (3). This is easily demonstrated by high-resolution polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (3). Similar treatment of materials prepared from *tolF* strains shows a marked difference in protein composition of the outer membrane when compared with material prepared from the wild-type strain. *tolF* and *cmlB* strains have much less of outer membrane protein 1 [nomenclature of Schnaitman (18)] (Foulds and Chai, manuscript in preparation).

At present, the inability to transfer the *tolD* mutation by transduction cannot be explained. Since Hfr strain G11e1 (*tolD*) can transfer colicin tolerance by conjugation, perhaps this strain contains an additional genetic alteration required for expression of the *tolD* phenotype. If so, locating the *tolD* locus by gradient mating techniques (2) would result in an apparent decreased inheritance of the *tolD* gene, placing it further away from the origin of transfer than it actually is.

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