Genet. Res., Camb. (1972), **19**, *pp.* 305–312
With 3 text-figures
Printed in Great Britain

Genetic locus determining resistance to phage BF23 and colicins E_1, E_2 and E_3 in *Escherichia coli**

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(*Received* 19 *April* 1972)

SUMMARY

The gene for resistance to phage BF23 and colicins E_1, E_2 and E_3, *bfe*, was mapped by a combination of conjugation and transduction crosses. Co-transduction of *bfe* was found with markers in the region between 76 and 79 min on the *Escherichia coli* genetic map. The highest frequency of co-transduction was found with *argH* (47 %). Three-factor transductional crosses showed unambiguously that *bfe* lies between *argH* and *supM*, at about 77·5 min on the map.

1. INTRODUCTION

Mutants of *Escherichia coli* that are not sensitive to the E group of colicins fall into two classes: colicin-resistant and colicin–tolerant. The initial distinction between these two classes was made on the ability of the colicin-insensitive mutants to adsorb the E colicins. Colicin-resistant mutants are altered in their cell surface so that they cannot adsorb colicin. Colicin-tolerant mutants still adsorb colicin but are not killed, presumably because of some block in the 'transmission' from the externally adsorbed colicin to the internal colicin-sensitive lethal site (Nomura, 1964; Nomura, 1967; Nomura & Witten, 1967; Hill & Holland, 1967; Nagel de Zwaig & Luria, 1967).

Bhattacharyya *et al.* (1970) demonstrated that colicin E-resistant mutants are altered in their cell walls, whereas a colicin E_1-tolerant mutant (*tolC*) is altered in its cell membrane. This was done by preparing membrane-vesicle ghosts, which are missing cell-wall materials (Kaback, 1971) from sensitive, tolerant and resistant strains. The active transport of proline in membrane ghosts from colicin-sensitive cells is inactivated when they are treated with colicin E_1, showing that the cell receptor for colicin E_1 remains on the ghosts and must therefore be on the cell membrane. Proline transport in ghosts prepared from resistant cells is also inactivated by treatment with colicin E_1, thus showing that the removal of something (the cell wall) provides access to the underlying membrane receptors.

The purpose of this report is to establish the genetic locus of the gene leading to

* Supported by research grants AI 08062 and FR 06115 from the National Institutes of Health. P. J. was a predoctoral trainee on National Institutes of Health training grant GM 00714.

colicin E-resistance. Mutants which are resistant to the otherwise unrelated colicins E_1, E_2 and E_3, as well as to bacteriophage BF23, were among the early genetic markers in *E. coli* (Fredericq & Betz-Bareau, 1952; Jenkin & Rowley, 1955). Reeves (1966), who called this gene *recE*, determined that colicin E resistance is closely linked to *argE* (then called *argA*) in conjugation experiments. In an abstract (Pfaff & Whitney, 1971) we renamed the gene *cer* for colicin *E* resistance, and it was subsequently referred to as such by Hull & Reeves (1971). Buxton (1971) independently mapped the same gene by conjugation and found no substructure for the locus in complementation tests. Buxton (1971) renamed the gene again: this time *bfe* for resistance to phage *BF*23 and the *E* group of colicins. To avoid further confusion we have adopted the notation *bfe*. Since we had utilized a *bfe* mutant in our studies of membrane-vesicle ghosts, we thought it worthwhile to precisely map the locus so it could be of use to those interested in cell-wall mutants and to those with other genetic markers in this region. The *bfe* gene has now been shown by three-factor transductional analysis to lie at about 77·5 min on the *E. coli* map, between *argH* and *supM*.

2. MATERIALS AND METHODS

The strains used and their relevant genotypes and sources are given in Table 1. The *bfe* mutant number 192 was isolated by Whitney (1970) from strain K10. It is resistant to colicins E_1, E_2 and E_3 as well as to phage BF23. As will be shown below, it can also be distinguished from tolerant strains in that *bfe192* cells do not adsorb colicin E_1.

Conjugational and transductional crosses were carried out by standard techniques as described previously (Whitney, 1970, 1971).

The scoring of unselected auxotrophic markers was by replica plating. Unselected *thi* markers were scored by replicating the transductants to be tested twice: first to a thiamine-deficient minimal medium plate and then (after a 48 h incubation at 37 °C) from that plate to another such plate. This was done to prevent the carry-over of a small but growth-supporting amount of thiamine from the original thiamine-supplemented plate to the thiamine-deficient plate. Rifamycin-sensitivity and resistance were scored by replica plating on tryptone-broth agar plates containing 100 μg rifamycin SV sodium salt per ml (from Swarz/Mann, Orangeburg, New York). After replicating from a tryptone broth 'master' plate to a rifamycin-containing plate, the replica was incubated for 90 min at 37 °C and then serially replicated to another rifamycin-containing plate. The process of 90 min incubation followed by serial replica plating was repeated once more, and after overnight incubation at 37 °C this final replica showed no growth for rif^s strains and heavy growth for rif^r strains.

The scoring of colicin-E-resistance (bfe^r or bfe^s) was done by overlays of plates stabbed with colicin-producing strains: broth agar in glass Petri dishes was stabbed in three or four places with sterile toothpicks dipped into overnight broth cultures of colicin E_1-, colicin E_2- or colicin E_3-producing strains. After overnight incuba-

Table 1. *Bacterial strains used*

Strain	Relevant genotype	Reference
AB1157	F⁻ *str*^{r} *proA thr leu his argE thiA*	DeWitt & Adelberg (1962)
DF1933	*arg*(*ECBH*) *metA*	Morrissey & Fraenkel (1969)
161	*metB arg*(*ECBH*)	Morrissey & Fraenkel (1969)
2587	*argH1 supM20 ilvD188* (*ilvD188* is an ochre mutation suppressed by *supM20*)	Eggertsson (1968)
2568	*purD38 thiA*	Eggertsson (1968)
P4X6R1	*metB rif*^{r}	Ezekiel & Hutchins (1968)
K10	*str*^{s} Hfr Cavalli prototroph	Whitney (1971)
K10 *bfe192*	*str*^{s} *bfe*^{r} prototroph	Bhattachrayya *et al.* (1970)
2568 *bfe1*	purD*38 thiA bfe*^{r}	this paper
CA38	(Col I, Col E_3)	Whitney (1971)
RC903	(Col E_1)	Whitney (1971)
RC906	(Col E_2)	Whitney (1971)
K10 *tolC65*	*tolC*	Whitney (1971)
K10 (Col E_1)	(Col E_1)	Whitney (1970)

Strains DF1933 and 161 were supplied by A. T. E. Morrissey, 2587 and 2568 by the Coli Genetic Stock Centre and P4X6R1 by D. Stroman. The other strains were from our laboratory collection or are derivatives of strains in this collection. Gene nomenclature is that of Taylor (1970); antibiotic and colicin sensitivity (ˢ) or resistance (ʳ) are denoted by superscripted letter symbols.

Table 2. *Frequency of unselected markers in cross of K10 Hfr str*^{s} *bfe192* × *AB1157 F*⁻ *str*^{r} *proA*⁻ *thr*⁻*leu*⁻ *argE*⁻ *his*⁻

Selected markers	Number tested	Unselected markers (%)				
		pro⁺	*thr*⁺*leu*⁺	*arg*⁺	*his*⁺	*bfe*^{r}
pro⁺ *str*^{r}	25	—	12	< 4	< 4	< 4
thr⁺ *leu*⁺ *str*^{r}	25	68	—	< 4	< 4	< 4
arg⁺ *str*^{r}	26	31	19	—	< 4	85
his⁺ *str*^{r}	26	15	8	19	—	19

tion at 37 °C the Petri dishes were sterilized by exposure to chloroform vapours (about 0·5 ml in the lid of an inverted Petri dish) for 45 min followed by venting for 1 h to allow the chloroform to evaporate. These plates were then overlaid with 3 ml of a soft agar (0·6 % Difco Bacto-Agar) containing 0·2 ml of an overnight broth culture. In early experiments the recombinants to be scored for *bfe*-resistance were tested against all three E colicins; in later experiments they were tested only with E_1.

Colicin E_1 was prepared by a modification of the mitomycin induction procedure of Maeda & Nomura (1966). The colicin E_1-producing strain was grown into late log phase in tryptone broth with added 0·01 M-$MgSO_4$. One μg mitomycin C per ml was added and after a further 4·5 h incubation at 37 °C the cells were concentrated 100-fold by centrifugation and resuspension in fresh tryptone broth. A few drops

Table 3. *Mapping of* bfe *by transduction : three factor crosses*

Selected donor marker (number scored)	Unselected markers	Co-transduction (%)	
Cross 1 *metA*$^+$ (118)	*bfe*^{r} *arg*(*ECBH*)$^-$	18	29
	bfe^r *arg*(*ECBH*)$^+$	11	
	bfe^s *arg*(*ECBH*)$^+$	1	12
	bfe^s *arg*(*ECBH*)$^-$	70	
Cross 2 *metB*$^+$ (120)	*bfe*^{r} *arg*(*ECBH*)$^-$	2	17
	bfe^r *arg*(*ECBH*)$^+$	15	
	bfe^s *arg*(*ECBH*)$^+$	12	27
	bfe^s *arg*(*ECBH*)$^-$	72	
Cross 3 *argE*$^+$ (149)	*bfe*^{r} *thiA*$^-$	23	45
	bfe^r *thiA*$^+$	22	
	bfe^s *thiA*$^+$	1	23
	bfe^s *thiA*$^-$	54	
Cross 4 *purD*$^+$ (114)	*bfe*^{r} *thiA*$^-$	3	37
	bfe^r *thiA*$^+$	34	
	bfe^s *thiA*$^+$	30	64
	bfe^s *thiA*$^-$	33	
Cross 5 *metB*$^+$ (124)	*bfe*^{r} *rif*^{r}	7	25
	bfe^r *rif*^{s}	18	
	bfe^s *rif*^{s}	1	19
	bfe^s *rif*^{r}	74	
Cross 6 *argH*$^+$ (130)	*bfe*^{r} *supM*$^-$	22	47
	bfe^r *supM*$^+$	25	
	bfe^s *supM*$^+$	1	26
	bfe^s *supM*$^-$	52	

In crosses 1 through 5, the donor strain was K10 *bfe192*; in cross 6 the donor strain was 2568 *bfe1*. The recipient strains in each cross were: 1, DF1933; 2, 161; 3, AB1157; 4, 2568; 5, P4X6R1; and 6, 2587.

of chloroform were added; and after shaking by hand and storage overnight at 4 °C the cells were removed by centrifugation. The supernatant fluid served as a crude colicin E_1 preparation and was frozen in 0·5 ml aliquots at −70 °C and thawed just prior to use.

To measure the adsorption of colicin E_1 and colicin titre in solution, late log-phase cultures of the *str*^{s} strains to be tested were concentrated tenfold by centrifugation and mixed at 37 °C with equal volumes of the crude colicin E_1 preparation. One ml samples were removed and centrifuged for 2 min at 15,000 rev/min in an International model MB Micro-centrifuge. The supernatant fluids (and the initial colicin E_1 preparation) were diluted in serial twofold steps and spotted (about 0·01 ml drops) on indicator plates overlaid with 3 ml of soft broth agar containing 0·01 ml of an overnight broth culture of a colicin-sensitive, streptomycin-resistant strain. The broth agar in the Petri dish contained 100 μg streptomycin per ml to kill any residual *str*^{s} cells in the fluid spotted. After overnight incubation at 37 °C, the last dilution to produce complete clearing of the lawn of indicator

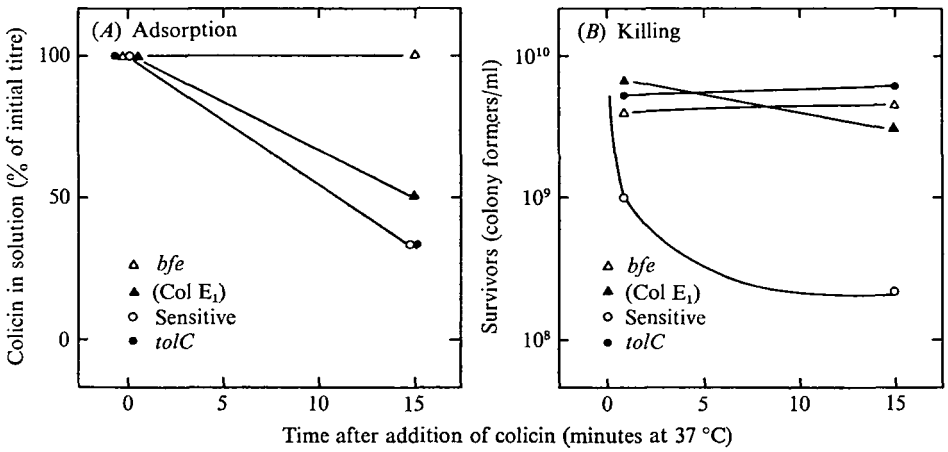

Fig. 1. Adsorption and killing by colicin E_1. Equal concentrations of colicin E_1 were incubated with sensitive (K10), *tolC* (K10 *tolC65*), *bfe* (K10 *bfe192*), and immune (K10 (Col E_1)) cells. Samples were withdrawn immediately and after 15 minutes and assayed for colicin remaining in solution (*a*), and for surviving colony-forming units (*b*).

bacteria was considered to contain one (arbitrary) colicin E_1 unit. Cell killing by colicin E_1 was assayed by diluting the exposed cells in 0·9 % saline and measuring surviving colony forming units on broth Petri dishes.

Colicin-resistant mutants were selected by streaking for single-colony isolation from the tiny colonies that appear in the inhibition zones in overlays of colicin-sensitive bacteria on plates containing chloroform-killed colicin E_1-producing bacteria. In general, about 50 % of such isolates are resistant to all three of the E-group colicins.

3. RESULTS AND DISCUSSION

First we confirmed the physiological characteristics of a colicin-resistant mutant. *Bfe192* is resistant to colicins E_1, E_2 and E_3 on stab plates and to phage BF23 (Whitney, 1970). The adsorption of colicin E_1 onto cells of the *bfe*^{r} mutant was tested in the experiment whose results are shown in Fig. 1(*a*). The sensitive strain and its otherwise isogenic derivatives that are mutant in the *tolC* locus or are colicinogenic (immune) for the E_1 factor adsorb 50–75 % of the colicin E_1 added. There is no detectable decrease in the free colicin titre on exposure to the colicin-resistant derivative, *bfe192*. In the same experiment, parallel samples were removed 1 and 15 min after the addition of colicin E_1 to the bacteria and assayed for surviving colony-forming units (Fig. 1*b*). Only the sensitive strain shows significant killing (about 90 % of the colony-forming units are lost). The conclusions from this experiment are that (i) the *bfe*^{r} mutant neither adsorbs colicin E_1 nor is it killed by the colicin, (ii) the *tolC* mutant and the colicinogenic (immune) strain adsorb colicin but are not significantly killed, and (iii) only the sensitive strain both adsorbs colicin E_1 and is killed by its action.

Next we turned to the genetics of the *bfe* gene. In a cross between a streptomycin-

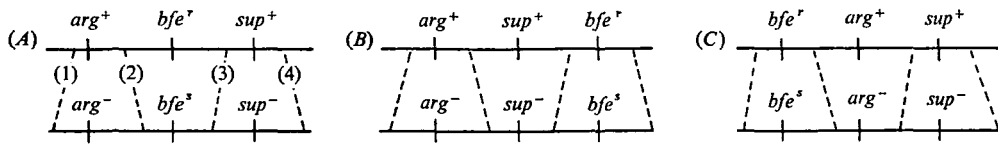

Fig. 2. Possible orders of the genes *argH*, *bfe*, and *supM*.

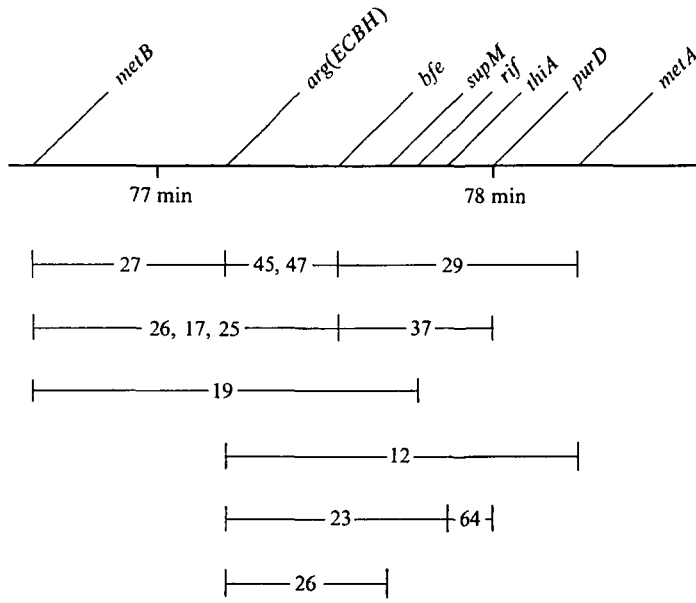

Fig. 3. Genetic map of the *bfe* region: co-transduction frequencies (%). The data are from Table 3 and the gene order from Taylor (1970) with the exception of *rif*, which was placed to the right of *supB* (probably identical to *supM*) and *supA* by Orias *et al.* (1972).

sensitive Hfr Cavalli strain (K10 *bfe192*) and a multiply-auxotrophic streptomycin-resistant F⁻ strain (AB1157), streptomycin-resistant prototrophic recombinants were selected and then scored for the other auxotrophic markers and for colicin E resistance or sensitivity. The results from this cross are shown in Table 2. *Bfe* shows close linkage in this cross to *argE* (85 % co-incorporation into recombinants), confirming the results of Reeves (1966) and Buxton (1971) in similar experiments with colicin-resistant strains.

Co-transduction of *bfe* with nearby markers on the *E. coli* map was tested by using P1 phage grown on a *bfe*-resistant donor (K10 *bfe192* or 2568 *bfe1*) to transduce auxotrophic recipients. Prototrophic transductants were selected, purified by restreaking once on minimal agar plates and then scored for colicin E resistance or sensitivity and for other unselected markers. The results (Table 3) show co-transduction of *bfe* with *metA*, *metB*, *arg(ECBH)*, *purD*, *rif* and *supM*, all of which are found in the region from 76–79 min on the Taylor (1970) map. The highest co-transduction (closest linkage, 45–47 %) is found with *argE* and *argH*.

https://doi.org/10.1017/S0016672300014555 Published online by Cambridge University Press

The *bfe* marker can be placed unambiguously between *argH* and *supM* on the basis of the three-factor transduction crosses whose data are given in Table 3. Consider the last cross in Table 3 (the one between 2568 *bfe1* and 2587). There are three possible gene orders: *bfe* may lie between the other two genes, to the right of sup*M* or to the left of *argH*(Fig. 2). The least-frequent class *argH*$^+$*bfe*^{s}*supM*$^+$, should arise from the rare occurrence of four crossing-over events. This is consistent with the first order of genes in Fig. 2. The three other classes of recombinants result from crossings-over in two of the four regions. The other two possible orders of the genes are inconsistent with the data. Hence we conclude that *bfe* lies between *argH* and *supM*. The map shown in Fig. 3 summarizes the data from Table 3 and places the *bfe* gene at about 77·5 min on the *E. coli* map (Taylor, 1970).

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