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# Genetic Analysis of *Escherichia coli* K12 Mutants Resistant to Bacteriophage BF23 and the E-Group Colicins

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*Summary.* A mutation conferring resistance to phage BF23 and the E-group colicins in *Escherichia coli* K12 has been mapped at a locus, designated *bfe*, between *arg*(*HBCE*) and *thi*. Mutants studied with this phenotype were recessive to wild-type and comprised a single complementation group.

Mutants of *Escherichia coli* K12 which are resistant to phage BF23 and form non-mucoid colonies are also usually resistant to the antibacterial proteins, colicins E1, E2 and E3 (Fredericq, 1949). These mutants are unable to adsorb both BF23 and the E-group colicins (Reeves, 1965), and have consequently been referred to as colicin-resistant (Jenkin and Rowley, 1955). They are quite distinct from colicin-tolerant mutants (Nomura, 1964), which retain their BF23 sensitivity and adsorb the E-colicins without being killed by them. Colicin-tolerant mutations are known to occur at a number of distinct loci (Taylor, 1970).

BF23-resistant mutants occur spontaneously at an approximate frequency of $10^{-6}$, and comprise about 70–80% of all mutants when selection is made for colonies resistant to an E-group colicin (Hill and Holland, 1967). Although the mutation in BF23-resistant cells is known to map near the *arg* gene cluster at position 77 on the *E. coli* map (Jenkin and Rowley, 1955; Reeves, 1966), its exact position has not been ascertained. In this paper, the location of the gene determining sensitivity or resistance to phage BF23 and the E-group colicins (*bfe*) is reported. A spontaneous $Bfe^R$ mutant was isolated in an $F^-$ strain (1638, see Table 1), and the site of the mutation was determined with respect to the *met*B, *arg*(*HBCE*) and *thi* loci in mating experiments with the Hfr strains P10 (orientation of transfer o-*argH-ilv*) and Reeves 4 (o-*pro-thr*). The three-point linkage data in Table 1 confirm the close linkage to *arg*(*HBCE*), and from the least frequent recombinant classes in the two crosses it is concluded that the gene order is *metB-arg*(*HBCE*)-*bfe-thi*. The data indicates a closer linkage of *bfe* to *arg*(*HBCE*) than to *thi*. Similar results were obtained in matings involving three other independently isolated $Bfe^R$ mutants.

Since this work was initiated, Pfaff and Whitney (1971) have also reported the map position of this gene. Their results are consistent with the above data.

In order to perform dominance and complementation tests, 7 independently isolated $Bfe^R$ mutants were obtained on the episome F14 present in the haploid strain AB1206 (F14/*pro his str* ▽ (*ilv-metB-argH*)), and 7 in a *recA1* derivative of 1638. The latter strain was constructed by selecting $His^+$ [$Str^R$] recombinants of 1638 after a 30 minute interrupted mating with strain KL16-99 (Hfr KL16

Table 1. *Location of the* bfe *locus*

| Selected recombinants | Phenotype | | | | Total |
|---|---|---|---|---|---|
| | $Arg^+$ $Bfe^S$ | $Arg^+$ $Bfe^R$ | $Arg^-$ $Bfe^R$ | $Arg^-$ $Bfe^S$ | |
| (a) $Met^+[Str^R]$ | | | | | |
| No. obtained | 270 | 12 | 26 | 1 | 309 |
| % of total | 87.4 | 3.9 | 8.4 | 0.3 | 100 |
| | $Thi^+$ $Bfe^R$ | $Thi^-$ $Bfe^R$ | $Thi^+$ $Bfe^S$ | $Thi^-$ $Bfe^S$ | |
| (b) $Arg^+[Str^R]$ | | | | | |
| No. obtained | 1 | 26 | 275 | 56 | 358 |
| % of total | 0.3 | 7.3 | 76.8 | 15.6 | 100 |

Cultures of the appropriate Hfr strain and a $Bfe^R$ derivative of the $F^-$ strain 1638 (*thr leu thi ilv metB arg(HBCE) pur his trp pro str*) growing exponentially in nutrient broth at 37° were mixed in the ratio of approximately 1 Hfr:5 $F^-$ for 1 hour. Samples were diluted and plated on appropriately supplemented minimal agar plates. After purification, colonies were classified with respect to unselected markers. In cross (a) the Hfr strain was P10 (*thr leu thi* $str^+$). In (b), it was Hfr R4 (*metB* $str^+$).

*recA1* $str^+$; orientation of transfer o-*thyA-his*), and screening for UV-sensitive colonies.

Merodiploids were then constructed by crossing AB1206 and its $Bfe^R$ derivatives with 1638 (carrying *recA1*) and its $Bfe^R$ derivatives. $Ilv^+$ $Met^+$ $Arg^+$ [$His^+$] progeny were selected from each cross, purified, and tested on the same selective medium for their resistance to phage BF23 and the E-group colicins. The heterozygotes in all cases were sensitive to BF23 and to colicins E1, E2 and E3. Consequently mutations conferring $Bfe^R$ are recessive to wild-type, whether present on the episome or on the chromosome. No complementation between any pair of $Bfe^R$ mutants was obtained, since all the pairwise combinations of $Bfe^R$ mutants were resistant to the agents used. These mutations conferring $Bfe^R$ can therefore be assigned to a single functional group.

Pfaff and Whitney (1971) have designated this locus *cer* (for colicin E resistance). I prefer the symbol *bfe*, since this conveys the additional information that these mutants are also resistant to BF23, in contrast to colicin E-tolerant mutants which are sensitive to this phage. Bhattacharyya *et al.* (1970) have shown that $Bfe^R$ mutants seem to retain a membrane receptor for colicin E1, since membrane vesicles prepared from such a mutant are still sensitive to this colicin. The defective wall component in these mutants may therefore prevent the colicin from gaining access to its membrane receptor, as Bhattacharyya *et al.* (1970) suggest. It is also possible that there are two colicin-specific receptors, one in the wall and one in the membrane. Similarly the component defective in these mutants may be a receptor for BF23, or concerned only with orientating the phage into the correct position for adsorption onto a membrane receptor.

Another class of colicin E-resistant mutants could be predicted: these would be unable to adsorb E-group colicins because of a change in a membrane receptor, but would presumably still be sensitive to BF23. Some of the mutants isolated by Hill and Holland (1967) may possibly be of this type.

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