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Genes Affecting Coliphage BF23 and E Colicin Sensitivity in *Salmonella typhimurium*

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Rough strains of *Salmonella typhimurium* were sensitive to coliphage BF23. Spontaneous mutants resistant to BF23 (*bfe*) were isolated, and the trait was mapped using phage P1. The *bfe* gene in *S. typhimurium* was located between *argF* (66% co-transducible) and *rif* (61% co-transducible). The BF23-sensitive *S. typhimurium* strains were not sensitive to the E colicins. Cells of these rough strains adsorbed colicin, as measured by loss of E2 or E3 killing units from colicin solutions and by specific adsorption of ^{125}I-colicin E2 to *bfe*$^+$ cells. Sensitivity to colicins E1, E2, and E3 was observed in a *S. typhimurium* strain carrying the F'8 *gal*$^+$ episome. This episome complemented the *tolB* mutation of *Escherichia coli*. We conclude that the *bfe*$^+$ protein satisfies requirements for adsorption of both phage BF23 and the E colicins. In addition, expression of a gene from *E. coli*, possibly *tolB*, is necessary for efficient E colicin killing of *S. typhimurium*.

A mutation of *Escherichia coli* that confers simultaneous insensitivity to phage BF23 and colicins E1, E2, and E3 was first described by Fredericq and is known as *bfe* (23). This mutation is located at min 79 on the *E. coli* chromosome (2, 11). Thus it is close to *rif*, the gene for the β-subunit of ribonucleic acid polymerase (1, 8). Mutants altered in the *bfe* gene are known as resistant strains, and the cells fail to adsorb the E colicins. They are distinguished on the basis of this property from several classes of tolerant (*tol*) mutants that adsorb colicins but are not efficiently killed (15, 16).

The product of the *bfe* gene has been solubilized from the outer membrane of *E. coli* (19) and has been characterized as a protein of molecular weight 60,000 by Sabet and Schnaitman (20). The partially purified receptor neutralizes colicins E2 and E3. It is not observed in a resistant mutant. This same protein is also involved in vitamin B_{12} binding prior to transport (3, 12). As a result of these studies, the product of the *bfe* gene is among the best characterized of *E. coli* outer membrane proteins genetically and in terms of cellular function and affinity for phage and colicins. The tolerance phenomenon, on the other hand, has not been well defined and is presumably concerned with a stage in colicin killing beyond adsorption.

It was our initial purpose to study the expression of the *bfe* allele from *E. coli* in the *Salmonella typhimurium* cell. We have constructed *S. typhimurium* merodiploid strains carrying the F'110 episome of *E. coli*. This episome spans min 79 and includes the alleles *bfe* and *rif*. We report here studies of the genetics of sensitivity of *S. typhimurium* strains to phage BF23 and another gene that affects E colicin sensitivity in this bacterium. These findings have been reported previously (S. K. Guterman, J. Lusk, D. H. Boyd, and A. Wright, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, K221, p. 183).

MATERIALS AND METHODS

Bacterial and phage strains. Phage strains were from the collection of this laboratory, except BF23 which was from S. E. Luria. BF23 was propagated on an *E. coli* K-12 strain.

The bacterial strains and sources are listed in Table 1. Strain GDA406 was derived from strain DB53 *cysA1348* (Am) *hisC527* (Am) *sup*$^+$ by P22 transduction from the donor GLA55. The population of transductants was enriched for *argA* cells with penicillin (14), samples were plated on minimal medium containing 1 μg of arginine per ml, and small colonies were screened for arginine auxotrophy. The *rif-4* gene was transferred from PMR4 by transduction. The *rec* gene was transferred from strain DB43 Hfr to a trimethoprim-resistant derivative of the arginine auxotroph by conjugation and selection for *thy*$^+$. The GDA406 strain and its derivatives in Table 1 are consequently *cysA hisC argA rec rif-4*. Merodiploid GDA406 strains containing F'110 and F'110Δ18 were constructed by conjugation and were selected as *arg*$^+$ exconjugants.

Rough mutants (24) were selected with phage P22

TABLE 1. *Bacterial strains*

Strain	Relevant characteristics	Source or reference
S. typhimurium LT-2[a]		
G30	*galE*	M. J. Osborn
GLS87	*argF87*	K. Sanderson
GLA55	*argA55*	B. N. Ames
DB43	*rec metA* HfrB2	D. Botstein
TA1659	Deletion: *bio chl-1013 dhb gal uvrB*[b]	B. N. Ames
SL1694	F'8 *gal*$^+$/TA1659[b]	B. A. D. Stocker
GDA406	*argA55, rec* from DB43 by conjugation	This study
GDA4061	P22^R from GDA406, Felix*h*^{R} rough[b]	This study
GDA4064	P22^R from GDA406, Felix*h*^{S}, rough	This study
GBS6	Str^R *bfe-11* from GDA4061	This study
SBE21	F'110/GDA406 from *E. coli* BE199	This study
SBE211	P22^R from SBE21, Felix*h*^{R}, rough	This study
SBC2	Spontaneous *argA55* from SBE211, presumed cured	This study
GFA61	F'110/GBS6 *bfe-11*	This study
GFA51	F'110Δ18/GBS6 *bfe-11*	This study
PMR4	*rif-4* ribonucleic acid polymerase mutation	This study
GPP3	P22^R, P1^S from PMR4 *rif-4*, rough	This study
GPA10	P22^R, P1^S from *argF87, bfe-10*, rough	This study
E. coli K-12[c]		
LE116	F'110/*metB recA56 argR*	J. Scaife, 6
LE114	F'110Δ18/*argR recA*	J. Scaife, 6
DB5	*metB1 argG6*	D. Boyd, 1
BE199	F'110/DB5 from LE116	This study
RVNal	Nal^R	M. Malamy
A437	*gal* Str^{R}F$^-$	S. E. Luria
A604	*tolB* from A437	S. E. Luria
Colicinogenic		
A796	(Col E1)	S. E. Luria
A798	(Col E2)	S. E. Luria
A617	(Col E3)	S. E. Luria

[a] Genetic symbols as in reference 21.

[b] The letters R and S indicate resistance and sensitivity. Drug- and phage-resistant clones were isolated as spontaneous mutants. Deletions are indicated by the symbol Δ. Strains with episomes are written as F'episome/recipient.

[c] Genetic symbols as in reference 23.

vir-3 or with both P22 *vir-3* and Felix*h*, which infects both rough mutants with complete core and wild-type smooth strains.

In the course of this work, phages T4 and T6 were used to distinguish *E. coli* from *S. typhimurium*. The rough strain *S. typhimurium* TA1659Δ*gal* was killed by T4 at a high concentration although plaque forming units were not obtained. Phage T6 has no effect on TA1659. The killing of certain rough strains of *S. typhimurium* by T4 has been independently observed in another laboratory (B.M. Tyler, personal communication).

Media and chemicals. LB broth and agar, soft agar, and OM minimal media have been described (7). Phage P1 was grown and titered in LB medium supplemented with 10 mM $CaCl_2$. Superbroth contained (per liter): tryptone, 32 g; yeast extract, 20 g; NaCl, 5 g; and 1 N NaOH, 5 ml. Mitomycin C, lactoperoxidase (B grade), and rifampin were obtained from Calbiochem. Carrier-free ^{125}I in 0.1 N NaOH was obtained from New England Nuclear Corp.

P1 transduction. Phage P1 from *E. coli* K-12 grew in *S. typhimurium* G30 *gal E* (18) with an efficiency of 5×10^{-4}, and high-titer lysates were prepared from these plaques. P1-sensitive mutants of PMR4 and GLS87 were obtained from rough mutants of each strain by cospreading with P1, incubating at 37 C, and screening for nibbled colonies. Phage P1 grown on donor GPP3 *rif-4* was used to transduce recipient GPA10 *argF87 bfe-10* (4).

Colicin preparation and iodination. Colicin was prepared from colicinogenic cells grown in superbroth at 37 C with aeration to early log phase, induced with 0.4 μg of mitomycin C per ml, and incubated 3 h at 37 C with aeration. The cells were collected and colicin was extracted and precipitated with ammonium sulfate as described by Herschman and Helinski (9). The protein concentrations of colicins E1, E2, and E3 were 18, 16, and 10 mg per ml, and the specific activities (9) were 9×10^4, 8×10^5, and 10^6 units/mg, respectively.

Colicin E2 was iodinated by a modification of the procedure of J. Carson (Ph.D. thesis, M.I.T., Cambridge, Mass., 1972). The reaction mixture contained 50 μl of ^{125}I (280 μCi) in 0.1 N NaOH, 50 μl of 0.1 N HCl, 10 μl of 0.1 M KI, 20 μl of 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.4), 50 μl of colicin E2, and 10 μg of lactoperoxidase. The reaction was started with 5 μl of 0.3% H_2O_2 and incubated at 0 C for 10 min. An additional 5 μl of H_2O_2 was added, and the reaction mixture was incubated 10 min longer. The reaction was terminated by exhaustive dialysis against 20% LB broth.

Colicin assay and adsorption. Dilutions of colicin were applied to a lawn of a sensitive indicator strain (*E. coli* K-12 RVNal) with an apparatus that can remove samples of 5 μl each from each of 25 sterile compartments. The titer was derived from the greatest dilution producing a clear zone of killing. Adsorption of unlabeled colicin was determined by incubating for 1 h at 37 C dilutions of colicin with equal volumes of adsorbing cells at an optical density at 600 nm of 1 in LB broth. Unadsorbed colicin was titered using strain RVNal on plates containing 50 μg of nalidixic acid per ml.

RESULTS

Sensitivity of S. typhimurium to phage BF23. The smooth F'110 merodiploid strain *S. typhimurium* SBE21 was partially sensitive to phage BF23 (Table 2); a sample of undiluted phage suspension spotted on a lawn of these cells resulted in a turbid area, and a sample of 100-fold diluted phage suspension produced a few small turbid plaques. The haploid parent strain GDA406 was totally insensitive. Rough mutants (24) derived from each of these strains, however, were sensitive to BF23. The most dilute suspension produced clear plaques on a lawn of strain SBE211 and a few small plaques on strain GDA4061.

We propose that cells of wild-type *S. typhimurium* have receptors for phage BF23 and possibly the E colicins and that these receptors are masked by the smooth O antigen. Colonies of mutants insensitive to BF23 were visible in the area of killing by undiluted phage on the lawn of rough *S. typhimurium* strain GDA4061. These BF23-insensitive mutants were tentatively labeled bfe_s^-, a subscript indicating the origin of the allele *S. typhimurium* or *E. coli* for the purpose of this study.

We constructed strain GFA61, merodiploid F'110 bfe_e^+/*S. typhimurium* bfe_s^-, to determine whether the *E. coli* allele bfe_e^+ can complement the bfe_s^- mutation in the *Salmonella* membrane to restore BF23 sensitivity. Table 3 compares the efficiencies of plating of phage BF23 on several rough *S. typhimurium* hosts with that in *E. coli* K-12. The bfe_s^- mutant did not yield any plaques, even with the greatest concentration of phage. The bfe_e^+ allele on the F'110 episome conferred an efficiency of plating of 14 to 22% to *S. typhimurium* bfe_s^- and bfe_s^+ strains, respectively, and the haploid bfe_s^+ strain SBC2 was 2.5% as efficient as was *E. coli* K-12 in plating phage BF23. Strain GFA51 bfe_s^- carrying the F'110Δ18 deletion episome and hence lacking the bfe_e^+ allele was not BF23 sensitive.

We conclude that rough *S. typhimurium* has a receptor for phage BF23 and can support vegetative growth of this phage at an efficiency

TABLE 2. *Sensitivity to coliphage BF23*

Strain	Relevant genotype	Phage sensitivity[a]			
		Undiluted	1:10²[b]	1:10⁴	1:10⁶
S. typhimurium					
GDA406	Smooth	−	−	−	−
GDA4061	Rough	+++	+++	+++	±
SBE21	F'110/smooth	+	±	−	−
SBE211	F'110/rough	+++	+++	+++	+++
E. coli					
B		+++	+++	+++	+++

[a] Samples of phage suspensions (titer 7 × 10⁹, undiluted) were applied to a soft-agar lawn of each strain on LB agar. Symbols: +++, clear area of lysis or large clear plaques; + and ±, small plaques or turbid area; −, no effect.

[b] BF23 dilution.

TABLE 3. *Efficiency of plating of BF23: complementation by E. coli bfe allele*

Strain	Relevant genotype[a]	BF23 titer	Efficiency of plating[b]
E. coli K-12			
DB5	bfe_e^+	6.7×10^9	1.00
BE199	F'110 bfe_e^+/DB5 bfe_e^+	6.2×10^9	0.93
S. typhimurium rough strains			
SBC2	bfe_s^+	1.7×10^8	0.025
SBE211	F'110 bfe_e^+/bfe_s^+	1.5×10^9	0.22
GBS6	bfe_s^-	$\leq 10^2$	$\leq 10^{-7}$
GFA61	F'110 bfe_e^+/bfe_s^-	9.2×10^8	0.14
GFA51	F'110Δ18 bfe_e^-/bfe_s^-	$\leq 10^2$	$\leq 10^{-7}$

[a] The *S. typhimurium* and *E. coli* alleles for *bfe* are distinguished by subscripts s and e, respectively.

[b] The titer of phage BF23 on each strain was normalized to that on *E. coli* DB5.

suggesting that BF23 is not restricted. The outer membrane protein of the *E. coli* allele bfe_e^+ can function in the *S. typhimurium* cell wall.

The bfe_s gene is co-transducible with rif. To determine whether the bfe_s allele of *S. typhimurium* is located in an analogous position to that on the *E. coli* chromosome, we used P1 transduction to determine whether this gene is linked to the markers *argF87* and *rif-4*. The mutation *rif-4* alters the β-subunit of ribonucleic acid polymerase and confers rifampin resistance to the enzyme in vitro (B. Young et al., manuscript in preparation). Transduction with phage P1 was necessary in this system since P22, the commonly used generalized transducing phage of *S. typhimurium*, requires the complete smooth O antigen as a cell wall receptor (24).

We constructed P1-sensitive strains GPP3 *rif-4* for the donor and GPA10 *argF87 bfe-10* for the recipient. A sample of GPA10 cells infected with P1 grown on GPP3 was plated on minimal medium lacking arginine. Another sample was diluted, grown for several generations, and plated on medium containing rifampin. The distribution of the unselected markers among transductants is shown in Table 4. Arg^+BF23^R Rif^R is the phenotype of the least numerous class of transductants. This must result from a double recombination event (four cross overs) and indicates that *bfe* is the middle marker.

TABLE 4. *Phenotypes of transductants in three-point argF, bfe-10, rif-4 cross*[a]

Selected marker	Phenotypes for unselected markers	No. obtained
Arg^{+b}	Rif^R $BF23^S$	39
	Rif^S $BF23^R$	27
	Rif^R $BF23^R$	4
	Rif^S $BF23^S$	21
Rif^{Rc}	Arg^+ $BF23^S$	33
	Arg^- $BF23^R$	35
	Arg^+ $BF23^R$	11
	Arg^- $BF23^S$	39

[a] The donor was GPP3 *rif-4*, and the recipient was GPA10 *argF87,bfe-10*

[b] Arg^+ transductants were cloned once on the same selective medium and then replica plated to this medium containing 20 μg of rifampin per ml, to control LB medium, and to LB medium spread with BF23.

[c] The transduction mixture was diluted and grown to allow expression of Rif^R genes. Transductants on selective medium were replica plated directly to media lacking arginine, to control medium, and to LB medium spread with BF23.

Individual co-transduction frequencies were obtained for each pair of genes from these data (Fig. 1). The gene order in *S. typhimurium* is *argF—bfe—rif*, the same as the order of the equivalent genes in *E. coli* (2, 11).

Colicin adsorption by bfe_s^+ strains. We examined the *S. typhimurium* smooth and rough strains for sensitivity to colicins E1, E2, and E3 by the soft-agar response test (15) and observed that all were insensitive by this criterion. Since the data in Tables 2 and 3 indicate that *S. typhimurium* has a phage BF23 receptor that is genetically similar to that of *E. coli*, it was of interest to determine whether the bfe_s^+ gene confers ability to adsorb colicins.

Adsorption tests performed with unlabeled or iodinated colicin E2 (Table 5) indicate that the bfe_s^+ or $F'bfe_e^+$ rough *S. typhimurium* strains adsorbed quantities of colicin E2 similar to *E. coli* K-12 strains. The bfe^- strains did not adsorb at all. Hence, E2 adsorption in *S. typhimurium* depends on the presence of a wild-type receptor protein. Similar specific adsorption to bfe^+ *S. typhimurium* strains was observed for colicin E3 (data not shown).

Genes on the F'8 gal^+ episome affect colicin sensitivity in S. typhimurium. A plausible hypothesis for adsorption of the E colicins without killing is that *S. typhimurium* is functionally tolerant. Since several *tol* genes map close to *gal* in *E. coli*, we tested strain SL1694 F'8 gal^+/ TA1659Δ*gal* for colicin E2 sensitivity by the soft-agar response test. We observed sensitivity although the circumference of the zone of killing was significantly smaller than that of a colicin-sensitive *E. coli* strain. A small proportion of gal^+ clones of SL1694, however, were not colicin sensitive. Gal^- derivatives of SL1694 that had presumably lost the episome and the parent TA1659 were not sensitive in this test. Several colicin-insensitive colonies obtained from within the clear area of killing of SL1694 were Gal^-; i.e., they had lost the F'8 episome.

Spot tests with concentrated colicin solution confirmed this finding and demonstrated that SL1694 was, in fact, more sensitive to colicins E1, E2, and E3 than was TA1694 (Table 6). The survival of these strains in the presence of colicin E2 was determined quantitatively (Fig. 2) and confirmed the spot test data; SL1694 was more sensitive (1.2% survival at 160 μg of E2 per ml) than was TA1659 (11% survival at the same concentration). The survival of *S. typhimurium* strain SBE21 carrying the episome F'110 was totally unaffected by any of the E colicins, even at the greatest concentration. By comparison, 0.0023% of the sensitive *E. coli* A437 cells survived treatment with 160 ng of E2, and 1.3%

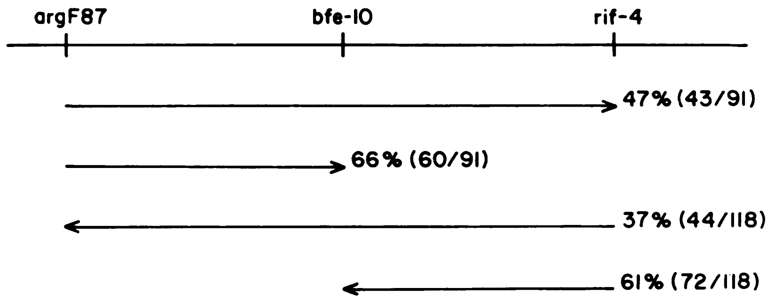

FIG. 1. *Map of bfe locus at min 128 in S. typhimurium. The base of the arrow indicates the selected marker, and the head indicates the unselected gene. The percent co-transduction frequency by phage P1 is calculated from the fraction (in parentheses) of total Arg$^+$ or Rif^R transductants acquiring the phenotype of the donor for the unselected trait (data from Table 4).*

TABLE 5. *Adsorption of E2: complementation by bfe_e^+*

	Expt 1		Expt 2	
Adsorbing cells and genotype	Relative colicin titer remaining[a]	Colicin adsorbed (%)	^{125}I counts/min on washed cells[b]	Specific adsorption[c]
None (LB broth)	64	0	3,230	
E. coli				
DB5 bfe_e^+	4	94		
BE199 F'110 bfe_e^+/bfe_e^+	4	94		
S. typhimurium				
GDA4061 bfe_e^+	16	75	11,360	8,130
SBE211 F'110 bfe_e^+/bfe_e^+	1	98		
GBS6 bfe_e^-	64	0	2,710	0
GFA61 F'110 bfe_e^+/bfe_e^-	4	94	10,930	7,700
GFA51 F'110Δ18 bfe_e^-/bfe_e^-	64	0		

[a] The colicin titer determined by spot test (see text) was normalized to eliminate fractions.

[b] ^{125}I-E2 (32,000 counts/min input) was added to 4 ml of cells at an optical density at 600 nm of 1, and the mixture was incubated for 1 h at 37 C with aeration. Cells and controls were washed by centrifugation, resuspended, and precipitated with an equal volume of 10% trichloroacetic acid, and the precipitates were collected on glass fiber filters and counted for radioactivity.

[c] Values were corrected for control without cells.

TABLE 6. *Sensitivity to colicins E1, E2, and E3 by spot test*

Strain	Relevant phenotype	Colicin titer		
		E1	E2	E3
E. coli				
A437	Colicin-sensitive	10^2	10^4	10^4
A604	*tolB* from A437	10^2	10^2	10^2
S. typhimurium				
SL1694	F'8 *gal*$^+$/TA1659	10^2	10^2	10^1
TA1659	Δ*gal*	0	≤1	≤1[a]
SBC2	*gal*$^+$ rough F$^-$	0	≤1	≤1[a]

[a] Turbid areas of killing were observed with undiluted and 1:10 dilutions of colicins E2 and E3.

of the cells of A604 *tolB* survived with 16 μg of E2 per ml.

The E2 colicin killing of the *S. typhimurium* strains does not display "one-hit" kinetics, as is seen by the plateau for SL1694 and the actual increase in survival of TA1659 at high E2 concentrations. These kinetics are not understood and may result from the presence of a colicin inhibitor or inactivator detectable only at high colicin concentrations. In the case of strain SL1694, there may be two populations of cells since colicin-insensitive *gal*$^+$ clones have been observed. The observation of Nomura and Witten that F'8 *gal*$^+$ episome did not complement *tol* mutations and our observation that some *gal*$^+$ clones of SL1694 were not colicin sensitive may be due to frequent deletions of *tol*$^+$ genes on this episome and enrichment of such clones, especially in an *S. typhimurium* background.

The tolB$^+$ allele is carried by the F'8 episome in strain SL1694. Several groups have demonstrated that *tol*$^+$ genes are carried on F'*gal*$^+$ episomes, such as F'1 (15, 16). However, the F'8 *gal*$^+$ episome studied by Nomura and

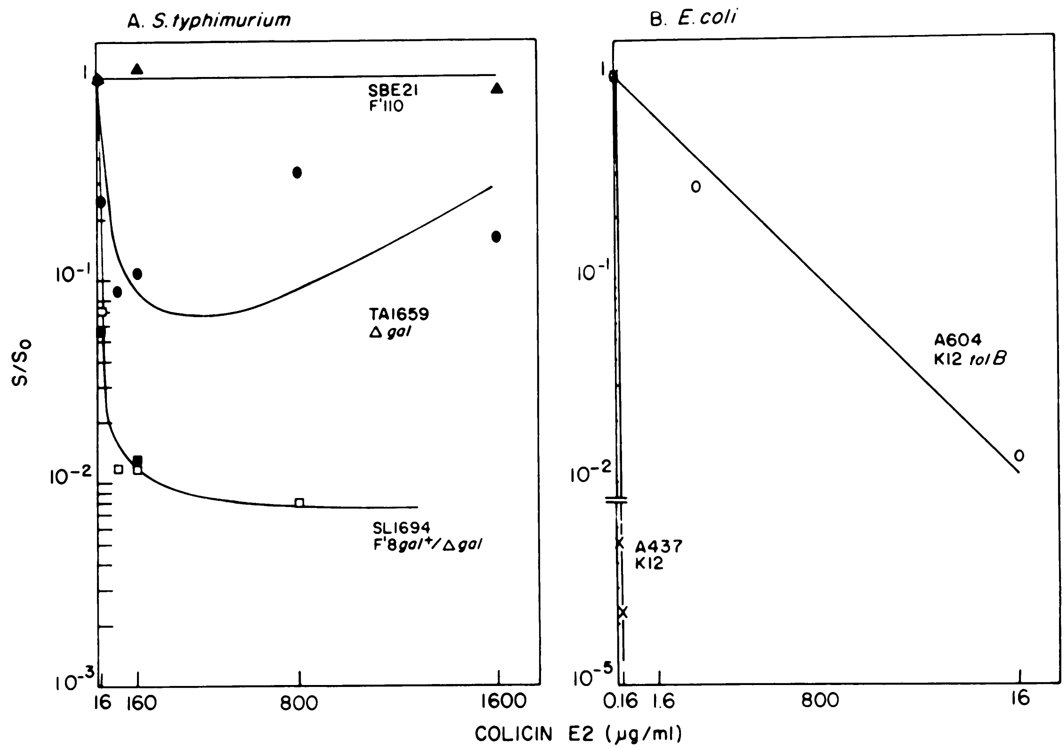

FIG. 2. *Killing of S. typhimurium strains as a function of colicin E2 concentration. Cells grown in LB broth at 37 C to a titer of 5 × 10⁷ to 5 × 10⁸ cells/ml were incubated with colicin E2 or control broth. Ater 10 min at 37 C, the mixtures were diluted 1:100 into cold LB broth and then diluted further and plated for survival. The fraction of surviving cells, S/S_0, is plotted as a function of the colicin concentration. The open and closed symbols are from separate experiments.*

Witten did not complement tolerance mutations. To determine whether a wild-type *tol*$^+$ gene was carried by the F'8 episome in SL1694, this strain was mated with *E. coli* strain A604 *tol gal strA*. The *gal*$^+$ *strA* exconjugants proved to be as sensitive to E2 as wild-type, colicin-sensitive *E. coli* strain A437, yielding titers three orders of magnitude greater than that on strain A604 *tolB*. We conclude that the F'8 *gal*$^+$ episome in *S. typhimurium* SL1694 confers colicin sensitivity to *S. typhimurium* and to *E. coli tolB*; hence it carries the wild-type allele for the *tolB* mutation.

DISCUSSION

The sensitivity of rough *S. typhimurium* to phage BF23 demonstrates that the O antigen lipopolysaccharide masks a phage receptor protein in the outer membrane. In rough strains the *bfe*$^+$ gene of *S. typhimurium* confers a phenotype similar to that of the *E. coli* counterpart: adsorption of the E colicins and sensitivity to phage BF23. The *E. coli bfe*$^+$ gene on the F'110 episome functions in the *S. typhimurium* outer membrane, suggesting that the *bfe*$^+$ proteins of these organisms are similar.

The *bfe* alleles in the two species are located between genes for arginosuccinase and the β subunit of ribonucleic acid polymerase (21, 23). The map distance by P1 transduction between *argH* and *bfe* in *E. coli* is 47% (11), compared with a distance of 66% for the equivalent genes in *S. typhimurium*. The map distance was determined in *E. coli* with the deletion of *arg(ECBH)* and in *S. typhimurium* with the point mutation *argF87*, so the data are not strictly comparable. The percent cotransduction of genes in *S. typhimurium* by P1 is greater than with P22. The *argF87—rif-4* distance reported here for P1 transduction is 37 to 47% compared with 9 to 23% observed using P22 (B. Young, personal communication). Such differences have been observed by Enomoto and Stocker (4).

We have shown that sensitivity of *S. typhimurium* to colicins E1, E2, and E3 is increased with additional genetic information carried on the episome F'8 *gal*$^+$. Several arguments rule against genes of F'8 *gal*$^+$ affecting the sensitivity of the biochemical targets of these colicins. Each of the E colicins has a characteristic effect on the cell, suggesting that the targets are different in each case (see Luria [13] for a review of this point). The F'8 gal^+ episome is relatively small and is unlikely to carry genes affecting the structures of such divergent macromolecules as ribosomal ribonucleic acid, deoxyribonucleic acid, and the energy-associated proteins of the membrane. The finding of Sidikaro and Nomura (22) of in vitro sensitivity of ribosomes of *Azotobacter vinelandii* and *Bacillus stearothermophilus* to colicin E3 suggests that ribosomes of *S. typhimurium* may be similarly sensitive.

It is more likely that the F'8 gal^+ episome carries a gene that affects the action of colicins at some stage subsequent to adsorption. We have shown that the F'8 episome used in this study includes the gene $tolB^+$. It may also carry $tolA^+$ or other genes that map close to *gal* and affect the tolerant phenotype. Since the *S. typhimurium* F'8 gal^+ strain is sensitive to the E colicins and the F'110 strain is not, F-specific genes are not responsible for this effect. The extent of sensitivity of the F'8 gal^+ *S. typhimurium* strain is less than that of a *tolB E. coli* strain. One may speculate that additional alleles from *E. coli* would be required to make colicin killing of *S. typhimurium* as efficient as it is in a fully sensitive *E. coli* strain.

Some classes of tolerant mutants of *E. coli* have defects associated with the cell wall or membrane that result in sensitivity to dyes and detergents (15). A *tolE* mutation (5) has been characterized that alters lipopolysaccharide sugars, and the phenotype can be suppressed by addition of galactose to the medium. These findings have led to speculation that tolerance to colicins may result from nonspecific alterations in the inner or outer membrane. However, rough *S. typhimurium* adsorbs E colicins but is not efficiently killed. It is tolerant in a natural state, and its membranes are not unusually fragile or defective. The degree of tolerance is reduced severalfold in the strains carrying F'8 gal^+.

Recently Jakes and Zinder (10) have found that pure colicin E3 consists of a complex of two proteins, an in vitro activity protein and an immunity protein. These are dissociable only by strong denaturing agents such as sodium dodecyl sulfate or guanidine hydrochloride. Although the immunity-free colicin has enhanced in vitro activity, it is not bactericidal. Ohsumi and Imahori (17) have characterized a factor from *E. coli* cytoplasm that enhances the in vitro activity of colicin E3. This factor may be a protease that removes the immunity substance since limited trypsin digestion also activates E3. The factor may be localized in the membrane in vivo and released by sonic treatment in their procedure. Their studies suggest that colicin E3 molecules may be "processed" by removal of the immunity protein after colicin adsorption.

We are currently determining the fate of colicin molecules in sensitive cells. Future studies with tolerant mutants may be of value in elucidating the structural membrane requirements of colicin killing.

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