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Bacteriophage Resistance in *Escherichia coli* K-12: General Pattern of Resistance

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Resistant mutants were isolated to 42 virulent bacteriophages in one strain of *Escherichia coli* K-12 and tested for resistance or sensitivity to a set of 56 bacteriophages. Most of the mutants fell into 11 groups with respect to their resistance patterns. It was possible to classify the bacteriophages broadly, according to the variety of mutants that were resistant to them.

Bacteriophage-resistant mutants are generally resistant to only some of the bacteriophages capable of forming plaques on the sensitive parent strain. Demerec and Fano (16), working with the set of seven T bacteriophages and *Escherichia coli* B, showed that a mutation could impart resistance to a single bacteriophage or simultaneously to several. However, this and other studies on cross-resistance in *E. coli* were limited to a small number of bacteriophages (15, 16, 23, 26, 34).

In this study we used 42 different virulent bacteriophages to isolate resistant mutants in *E. coli* K-12 and report the results of a survey of the cross-resistance patterns of these mutants.

Most bacteriophage-resistant mutants that have been characterized are receptor mutants and this study was undertaken because, as one might expect by analogy with studies on colicin-resistant mutants (9, 10, 27, 29), such a survey could help define the variety and specificity of bacteriophage receptors. It is also hoped that the mutants isolated in this study will provide a tool for further studying the various components of the cell wall of *E. coli* K-12.

MATERIALS AND METHODS

Bacteria. The parent strain P400 (*thr ara leu proA lacY galK non xyl mtl argE thi str* λ^- *sup-37* [amber]) was an *E. coli* K-12 derivative (30).

Bacteriophages. The bacteriophages used in this study and their sources are listed in Table 1. In some cases bacteriophages have been given laboratory strain names, either for the sake of simplicity or due to similarity of names in the literature. The previous names, where appropriate, and a literature reference to the bacteriophages are also listed in Table 1. Some of the phages were originally isolated on other strains of *E. coli* or *Shigella* strains. All have been reisolated three times on strain P400 from single plaques and propagated in liquid media (3), except in the case of the T set of bacteriophages, which were classically grown on *E. coli* B (15), and bacteriophages H1, H3, H8, and E11, which were also propagated on *E. coli* B, since these bacteriophages closely resembled the T bacteriophages (see Table 2).

Media. Nutrient broth (Difco 0003) was prepared double strength plus 0.5% (wt/vol) sodium chloride. Nutrient agar was blood agar base (Difco 0045) prepared as directed without the addition of blood. Brain heart infusion medium (Difco 0037-01), used in the isolation of bacteriophages from sewage, was prepared as directed; 0.7% agar for overlays was prepared by diluting nutrient agar 1:1 with nutrient broth.

Isolation of bacteriophages from sewage. The phage isolation technique used was that of Brown and Parisi (11), using raw sewage from the Bolivar sewage treatment farm near Adelaide. All bacteriophages were isolated on strain P400 or its parent strain, AB1133. Bacteriophages were isolated from three samples of sewage. Bacteriophages A, B, C, D, F, G, and J were isolated from one sample, while H1, H3, and H8 were isolated from a second sample. To increase the variety of bacteriophages obtained, antisera raised, as described below, against bacteriophages F, H3, T6, and T3 were used to select against bacteriophages of similar types during the third isolation; of the 25 bacteriophages isolated, E4, E7, E11, E15, E21, and E25 were chosen for further study.

Serology. Rabbit anti-bacteriophage sera were prepared by the three course immunization schedule of Barry (5). A single rabbit per bacteriophage was used to raise antisera against bacteriophages T6, H3, T3, and F, with K values of 712, 767, 157, and 606, respectively, in neutralization studies using the complementary bacteriophages. Rabbit anti-T4 antiserum (K = 659) was a gift of A. Osmand, and rabbit anti-T2 antiserum (K = 1091) was a freeze-dried preparation made some years ago by one of us (P.R.). The neutralization assay and method of determining K values were those of Barry (5), using bacteriophage solutions suspended in nutrient broth. A K value of 1 or greater in a neutralization assay was taken to indicate the serological relatedness of the bacteriophage used to prepare the antiserum and the bacteriophage used in the assay. Results are summarized in Table 2.

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TABLE 1. *Bacteriophage strains used*

Bacteriophages	Source[a]	Previous name[b]	References
T1, T2, T3, T4, T5, T6, T7	1		3
BF23	1		13
A, B, C, D, F, G, J, E4, E7, E11, E15, E21, E25, H1, H3, H8	2		
K2	3	IISK = SsII	21
K3	3	VSH = SHV	22
K4	3	VSK = SsV	21
K5	3	VISK = SsVI	21
K6	3	VIISK = SsVII	21
K8	3	IXSK = SsIX	21
K9	3	XSK = SsX	21
K10	3	XISK = SsXI	21
K11	3	XIISKo = SsXII	21
K12	3	XIISHo = SHXII	22
K15	3	G36 = SG36	20
K16	3	G42 = SG42	20
K17	3	T1881	32
K18	3	α	31
K19	3	a	31
K20	3	D8	31
K21	3	D2a	31
K22	3	D2b	31
K25	3	F2	31
K26	3	F4	31
K27	3	F5	31
K29	3	F7	31
K30	3	F9	31
K31	3	F10	31
Ox1, Ox2, Ox3, Ox4, Ox5	4		19
M1	4	Phage 3	19
M3	4	C1 = ϕ1	7
Ac3	5	3	2
Ac4	5	4	2
H^+, V	6		6
ϕI	7 and 10		17
H	7		12
ϕII-T	8		25
F27	9		33
W31	11		36

[a] Sources were: 1, lab stock; 2, isolated from sewage in this study; 3, S. Ślopek; 4, D. Kay; 5, M. W. Ackerman; 6, J. Beumer; 7, R. W. Hyman; 8, M. H. Malamy; 9, I. W. Sutherland; 10, R. Dettori; 11, T. Watanabe.

[b] Two alternatives have been given where more than one name exists in the literature.

When a bacteriophage was neutralized by either anti-T2, anti-T4, or anti-T6 antisera, the expression anti-T-even has been used, since T2, T4, and T6 are serologically related (3). In general, anti-T6 antiserum was used, sometimes in conjunction with anti-T2 and anti-T4 antisera. Although we did not conduct many detailed quantitative studies, results with the different antisera were similar for any given bacteriophage, except for bacteriophage H3. This bacteriophage provides an exceptional case since it is neutralized (K = 1.5 to 2.4) by anti-T6 antiserum but not by anti-T2 or anti-T4 antiserum. Furthermore, bacteriophage T6 is not neutralized by anti-H3 antiserum. Thus, bacteriophage H3 can be distinguished serologically from the other T-even bacteriophages.

Electron microscopy. Preparation of bacteriophages was by a technique based on that of Bradley (8). The bacteriophages were harvested from five overlaid plates (0.7% agar) showing semiconfluent lysis, by collecting the overlay into 3 ml of 1% (wt/vol) ammonium acetate (pH 7.0). They were then purified, concentrated by one cycle of differential centrifugation, and resuspended in a small volume of ammonium acetate buffer. Uranyl acetate was added at 2% (wt/vol), and the samples were examined at a magnification of $\times$33,000 on a Siemens-Elmiskop electron microscope.

Resistant mutant isolations. To isolate mutants of independent origin, we streaked individual colonies across nutrient agar plates on which 10^7 to 10^8 of the appropriate bacteriophage had been spread. From each streak a single colony was then picked off, purified by two successive single-colony isolations, and tested for sensitivity to the set of bacteriophages listed in Table 1. One or two of each type were selected for further study. All mutants isolated arose spontaneously with the exception of strain P479, which was isolated after mutagenesis of strain P400 by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by the method of Adelberg et al. (4).

Testing of resistance or sensitivity. Two methods were used to test resistance and sensitivity. The first was a general method and employed a multiple-syringe bacteriophage applicator based on that of Zierdt et al. (37). This device enabled us to test 100 or more mutants against 48 bacteriophages in less than a day with reproducible results and no contamination. Each drop made with the applicator contained 10^7 or 10^3 plaque-forming units of bacteriophage, both concentrations of each bacteriophage being used with each mutant tested. If a mutant was not fully resistant or sensitive to a bacteriophage, it was characterized by the second method, relative efficiency of plating (EOP). EOP was determined by plating 0.1-ml amounts of serial 100-fold dilutions of a given bacteriophage stock (10^9 plaque-forming units per ml) with 0.1 ml of an overnight culture of strain P400 or one of its bacteriophage-resistant derivatives. The relative EOP in this paper represents the number of plaques formed on a mutant relative to the number formed on the parent strain P400.

RESULTS

Electron microscopy of bacteriophages. Electron microscopy was performed on bacteriophages A, F, G, J, D, H1, H3, H8, E4, E7, E11, and F27. These bacteriophages were similar in morphology to others well described in the literature, and therefore individual electron micrographs are not presented in this paper. Bacteriophages T3 and T4 were included as standards.

Bacteriophages A, F, and J were P1-like (A1 in the Ackerman [1] nomenclature) and had the following dimensions: head, 92.2 $\pm$ 0.9 by 85.4

TABLE 2. *Bacteriophages, their taxonomic criteria, and the groups of mutants that are commonly resistant to them*[a]

Bacteriophages	Electron microscopy[b]	Serology[c]	Resistance groups[d]
E4	A1	—	Efr, Wrm, Bar, Misc
F27	A1	—	Ttk, Wrm, Bar, Misc
E7	A1	—	Ttk, Wrm, Bar, Misc
K19	A1	—	Ttk, Wrm, Bar
A, F, G, J	A1	Anti F	Wrm, Bar
H3	A2	Anti H3	Tsx
T6, H1, H8, K9, K18, K31, Oxl	A2	Anti T-even	Tsx, Wrm
K3, K4, K5, Ox3, Ac3	A2	Anti T-even	Con, Wrm
Ox2, Ox4, M1	A2	Anti T-even	Con, Wrm, Bar
Ox5	A2	Anti T-even	Con, Wrm, Bar, Ttk, Misc
T2	A2	Anti T-even	Ttk, Wrm
T4	A2	Anti T-even	Ttk, Wrm, Bar, Misc
K2	A2	Anti T-even	Ktw, Wrm, Bar
K20, K21	A2	Anti T-even[e]	Ktw, Wrm, Bar
K29	A2	—	Ktw, Wrm, Bar
T5, D, E21[f]	B1	—	Ton A
T1, E25, K22, K26, K27, K30[g]	B1	—	Ton A, Ton B
BF23, E15, K6, K8, K11, K12, M3, Ac4[h]	B1	—	Bfe
K15	B1	—	Wrm
K25	B1	—	—
K10	B2	—	Ktn, Wrm
T3	C1	Anti T3	Bar, Wrm
K17	C1	—	Wrm, Bar, Misc
K16	C1	—	Ttk, Wrm, Bar, Misc
T7, φI, H, W31, E11	C1	Anti T3	Wrm
φII-T	C1	Anti T3	
H^+	—	—	Ttk, Wrm, Bar, Misc
V	—	—	Ttk, Wrm, Bar, Misc

[a] Details of minor variations in resistance pattern are included in Tables 3 through 9.

[b] Electron microscope appearance is as described by Ackerman (1): A1 (P1-like), long contractile tail, isometric head; A2 (T-even-like), long contractile tail, elongated head; B1 (T5-like), long noncontractile tail, isometric head; B2, long noncontractile tail, elongated head; C1 (T3-like), short noncontractile tail, isometric head; —, not done.

[c] Rabbit anti-bacteriophage antisera tested against each bacteriophage were anti-T2, anti-T4, or anti-T6 (anti T-even); anti-T3; anti-H3; and anti-F. Antisera with neutralizing activity are included in the table. —, Not neutralized by the above antisera.

[d] Mnemonics are the same as the genotypic mnemonics where one member of the group has previously been mapped. Con, Conjugational recipient deficiency (30); Ktw, K2 resistance; Wrm, wide-range mutants; Ktn, K10 resistance; Ttk, T2, T4, or K19 resistance; Bar, bacteriophage A resistance; Misc, miscellaneous group; Efr, E4 resistance.

[e] Only shows partial neutralization ($0.8 < K < 1.2$) with anti-T2 antiserum and anti-T6 antiserum (anti T4-antiserum not tested).

[f] T5, D, and E21 are similar with all criteria and are henceforth referred to as the T5-like bacteriophages.

[g] T1, E25, K22, K26, K27, and K30 are similar with all criteria and are henceforth referred to as T1-like.

[h] BF23, E15, K6, K8, K11, K12, M3, and Ac4 are similar with all criteria and are henceforth referred to as BF23-like.

± 1.6 nm; tail, 114.0 ± 1.9 by 21.3 ± 1.3 nm. Bacteriophages G, E4, and E7 were similar but with smaller heads: 82.3 ± 1.7 by 75.6 ± 2.0 nm. Bacteriophage F27 also had a morphology and tail size similar to the above bacteriophages but had an even smaller head: 72 by 67 nm. Bacteriophage D was T5-like (B1 in the Ackerman [1] nomenclature) with the following dimensions: head, 85 nm^2; tail, 198 by 10 nm. Bacteriophages H1, H3, H8, and T4 were of T-even morphology (A2 in the Ackerman [1] nomenclature) and had the following dimensions: head, 116.4 ± 6.9 by 86.4 ± 5.7 nm; tail, 110.9 ± 8.5 by 23.6 ± 2.0 nm. Bacteriophages E11 and T3 were similar morphologically (C1 in the Ackerman [1] nomenclature) and had the following dimensions: head, 52 nm^2; tail, 14 by 6 nm. Results are summarized in Table 2, to-

gether with results obtained from the literature. The complete set of 62 bacteriophages contained 8 of A1 morphology, 23 of A2 morphology, 19 of B1 morphology, 1 of B2 morphology, and 9 of C1 morphology.

Resistance types. Five different degrees of resistance were noted in this study. They are defined under the following headings, and the initials or symbols in parentheses are the abbreviations used in Tables 3 through 8.

(i) Full resistance. For the full-resistance (R) type, the bacteriophage is unable to form plaques on a particular mutant (EOP $< 10^{-7}$).

(ii) Bacterial inhibition. For the bacterial inhibition (I) type, the bacteriophage plaques are very turbid (i.e., bacterial growth is inhibited where a clear plaque would normally form, the area of inhibition being equal to the normal area of a plaque). Plaques occur with an EOP of 1.

(iii) Partial resistance. For the partial-resistance (P) type, the bacteriophage forms wild-type plaques with an EOP of 10^{-2} or less.

(iv) Partial resistance with inhibition. For the partial-resistance type with inhibition (IP), a combination of the above two effects occurred, with very turbid plaques and a lowered EOP.

(v) Slight resistance. For the slight-resistance (SL) type, the bacteriophage either forms wild-type plaques at an EOP of greater than 10^{-2}, or only minute plaques are formed with an EOP of approximately 1. These are very minor alterations in the resistance pattern and thus they have been grouped.

(vi) Sensitive. For the sensitive (-) type an EOP of approximately 1 with normal plaques resulted.

The terms resistance and resistant, where used in this paper, cover any of the above terms except for sensitivity. These terms are distinguished from full resistance or fully resistant as defined above.

Isolation of mutants. When bacteriophage-resistant mutants are selected in *E. coli* K-12 many of the mutants are mucoid, their resistance apparently being due to the physical barrier presented to bacteriophage attachment by a layer of capsular polysaccharide. This layer is formed in the single-step mutants *lon* or *capS* (24). These mutants were avoided by using a *non* strain, P400, as the parent strain for selection of bacteriophage-resistant mutants. The *non* mutant is blocked in capsular polysaccharide synthesis, and the *lon non* and *capS non* double mutants have the phenotype of the *non* single mutant (26). Thus, mucoid mutants are not picked up in this strain. It has been shown that the *non* mutation does not alter the composition of the lipopolysaccharide (R. Hancock and P. Reeves, manuscript in preparation).

Over 500 resistant mutants were isolated by using 42 different virulent bacteriophages to select the mutants. Between 20 and 200 apparent mutant colonies were selected with each bacteriophage used, although many, when purified, were found to be fully sensitive to all bacteriophages. Each mutant was tested against the full set of bacteriophages in use at that time so that about 400 were tested against all bacteriophages. Those selected for further study were carefully retested against all bacteriophages, and their biochemical markers were checked. All mutants had the same amino acid requirements as the parent strain P400 and, in addition, were unable to ferment galactose, lactose, mannitol, and xylose.

All bacteriophages used to select resistant mutants had clear-centered plaques and were thus assumed to be virulent. This was further confirmed by demonstrating that the resistant mutants were not lysogenic, since when the culture supernatant from 5×10^9 cells was plated with an indicator strain, AB1133, no plaques were observed. For some mutants the supernatant of a ultraviolet-irradiated culture was plated.

Resistance groups. The following resistance patterns arose.

(i) Ton A, Ton B, Bfe, Con, Efr, and Ktn groups. The mutants in groups Ton A, Ton B, Bfe, Con, Efr, and Ktn were fully resistant to all bacteriophages in the group (Table 3). Only representative bacteriophages are presented for the Ton A, Ton B, and Bfe groups since the bacteriophages to which they were resistant had very similar morphologies and were probably

TABLE 3. *Fully resistant mutants of the Ton A, Ton B, Bfe, Con, Ktn, and Efr groups of resistant mutants*

Phenotype	Isolated against	No. of mutants	Representative mutant	Resistant to[a]
Ton A	T1, D, E21, E25, K22, K26	32	P417	T1-like, T5-like
Ton B	T1, E25, K26	10	P442	T1-like
Bfe	E15, K8, K12	21	P445	BF23-like
Con	K3, K5	8	P460	K3, K4, K5, Ox2, Ox3, Ox4, Ox5, M1, Ac3
Ktn	K10	10	P466	K10
Efr	E4	10	P448	E4

[a] See Table 2 for full description of bacteriophages. Each mutant was tested against each of the bacteriophages used in this study, and every case of resistance is included in the Table.

just different isolates of the same bacteriophage, despite their very different origins (Table 1). Preliminary genetic and colicin resistance studies indicate that the Ton A, Ton B, and Bfe groups of mutants are identical to the *ton* A, *ton* B, and *bfe* mutants isolated previously (R. Hancock, J. Davies, and P. Reeves, manuscript in preparation).

(ii) Tsx group. Two subgroups of mutants were found in resistance group Tsx, which showed common resistance to a set of eight bacteriophages (Table 4). The most common mutant type (99%) is probably (Hancock, Davies, and Reeves, manuscript in preparation) the classical *tsx* mutant. The only mutant of subgroup 2 was fully resistant to bacteriophages H1, H3, H8, and K18, and showed partial or slight resistance to the others.

(iii) Ktw group. Mutants in group Ktw are resistant to a set of four bacteriophages. Subgroups 2 and 3 (one mutant each) are distinguished from the other mutants by cross-resistance to bacteriophages E4 and H^+, respectively (Table 5). The mutants in subgroup 1 are resistant (to varying extents) only to the four bacteriophages.

(iv) Ttk group. Group Ttk is less well defined than the previous groups. Common resistance to bacteriophages Ox5, K16, F27, H^+, and V (Table 6) has been used to justify the grouping of these mutants. They are differentiated into subgroups according to their pattern of

TABLE 4. *Bacteriophage resistance of the Tsx group of mutants*

Subgroup	Representative mutant	Isolated against	No. isolated	Resistance[a]							
				H3	T6	H1	H8	K9	K18	K31	Ox1
1	P407	T6, H1, H3, H8, K18	97	R	R	R	R	R	R	R	R
2	P433	H1	1	R	SL	R	R	P	R	P	SL

[a] All abbreviations as in the nomenclature section of Results. Each mutant was tested against each of the bacteriophages used in this study, and every case of resistance is included in the Table.

TABLE 5. *Bacteriophage resistance of the Ktw group of mutants*

Subgroup	Mutants	Isolated against	Resistance[a]					
			K2	K20	K21	K29	E4	H^+
1	P477	K20	IP	IP	IP	IP		
	P458	K2	IP	R	R	SL		
	P456, P457	K2	IP	R	R	R		
2	P476	K2	IP	R	R	R	SL	
3	P240	K29	SL	IP	IP	IP		I

[a] All abbreviations as in nomenclature section of Results. Each mutant was tested against each of the bacteriophages used in this study, and every case of resistance is included in the Table.

TABLE 6. *Resistant mutants of the Ttk group and miscellaneous (Misc) mutants resistant to between two and five of the bacteriophages to which Ttk group mutants are resistant*

Group	Subgroup	Mutants	Isolated against	Resistance[a]										
				Ox5	K16	F27	H^+	V	E7	K17	T4	K19	T2	E4
Ttk	1	P429	T2	P	R	R	R	R	—	—	—	R	R	R
	2	P423	T4	SL	R	R	R	SL	R	SL	R	—	—	—
	3	P425	T4	R	R	R	R	R	IP	—	R	R	—	—
		P432	T4	SL	R	R	R	R	IP	—	R	R	—	—
	4	P474	K19	P	R	R	R	R	—	—	—	R	—	—
Misc	1	P491	T4	—	SL	—	R	—	P	SL	P	—	—	—
	2	P443	E7	—	—	—	—	—	P	—	SL	—	—	—
	3	P498, P499, P238	F27, K16	—	SL	R	SL	—	—	—	—	—	—	—
	4	P237	F27	SL	SL	R	SL	—	—	—	—	—	—	—
	5	P493	K17	SL	SL	—	—	—	—	SL	—	—	—	I

[a] All abbreviations as in the nomenclature section of Results. Each mutant was tested against each of the bacteriophages used in this study, and every case of resistance is included in the Table.

resistance to bacteriophages T2, T4, and K19. Resistance to both T4 and K19 is found (subgroup 3) as well as resistance to one but not the other (subgroups 2 and 4). The bacteriophage T2-resistant mutant that comprises subgroup 1 differs from the others in that it is uniquely cross-resistant to bacteriophage E4. Some of the mutants are cross-resistant to bacteriophage E7 and one is also slightly resistant to bacteriophage K17.

(v) Miscellaneous group. The miscellaneous group is even less well defined than the Ttk group and includes five mutant types, which do not fit well into other groups. They are resistant to bacteriophages that are common to the Ttk group. Two are resistant to some extent to bacteriophage T4 (subgroups 1 and 2). Subgroup 3 (containing three identical mutants) and subgroup 4 (only one mutant) appear to be similar (Table 6).

(vi) Bar group. Nineteen mutants were sorted into eight subgroups (Table 7). The mutants were resistant to between 10 and 19 bacteriophages. Subgroups 1 and 3 were sensitive to T3, whereas subgroups 4 to 8 were resistant. Subgroup 1 was distinguished by its sensitivity to bacteriophage K16, whereas subgroup 2 was uniquely sensitive to bacteriophages A and E7. Subgroup 3 contained all the remaining T3-sensitive strains and showed, at least, resistance to a set of 12 bacteriophages. Of the T3-resistant strains, subgroup 4 was uniquely sensitive to bacteriophage K2. Subgroup 5 was found to be sensitive to H^+ and only slightly resistant to T3. Subgroup 6 was sensitive to bacteriophage K17 but resistant to T4 and K2. Subgroup 7 was resistant to all bacteriophages except K19, whereas subgroup 8 was resistant to this bacteriophage too. Although this group is extremely heterogeneous, it nevertheless seems to fit well as a group. It is interesting that nearly all of the bacteriophages to which the miscellaneous, Ktw, and Ttk groups are resistant are included in the set of bacteriophages to which Bar mutants are resistant.

(vii) Wrm group. Only one of the Wrm group mutants, P479, was selected by using a

TABLE 7. *Mutants of the Bar resistance group*

Subgroup	Mutants[a]	Resistance[b]																	
		M1	Ox2	Ox4	E4	K2	K20	K21	K29	Ox5	K16	F27	H^+	V	E7	K17	A	T3	T4	K19
1	P455		P	P	SL	SL	IP	IP	I	P					SL		SL			
2	P492	SL	P	R	R	I	R	R	I	R	IP	R		R		P				
3	P409		R	R	R	I	IP	IP	SL	R	R				P	SL	P			
	P413	P	R	R	R	IP	P	P	IP	R	R				P	SL	R			
	P404	P	IP	IP	R	IP	IP	IP	R	IP	IP	R			IP	P	IP			
	P495	SL	R	R	R	R	IP	R	R	R	P	R			R	P	R			
	P497	SL	P	R	R	I	R	R	R	R	R	R			R	P	R			
	P494	P	P	R	R	I	SL	SL	IP	R	P	R	R		R	SL	R			
	P496	P	P	R	R	I	IP	IP	IP	R	IP	R	R		R	SL	R			
	P415	P	P	R	R	I	IP	IP	R	R	P	R	I	R	R	P	R			
4	P490		P	R	R		IP	IP	IP	R	SL	R	R		R		R	R		
	P428	SL	P	R	IP		I		I	R	P	R	R		R		R	R		
	P405	SL	R	R	R		I	I	IP	R	P	R	R		R		R	R		
	P436	SL	R	R	P		SL	SL	I	R	SL	R	R		R		R	R		
5	P402	SL	I	R	R	R	IP	IP	I	R	I	R		R	R	R	IP	SL		
6	P451	SL	R	R	R	R	R	R	R	R	R	R	R		R		R	R	SL	
7	P487	SL	P	R	R	I	IP	IP	SL	R	P	R	R	I	R	SL	R	R	SL	
	P488	SL	R	R	R	I	R	R	I	R	P	R	R	R	R	SL	R	R	SL	
8	P489	SL	R	R	R	IP	R	R	R	R	R	R	R	R	P	P	R	R	P	R
		Con[c]			Efr[c]	Ktw[c]				Ttk[c]							Bar[c]		Ttk[c]	

[a] The mutants were selected by using the bacteriophages in parentheses: P455 (against K2), P492 (K17), P409 (J), P413 (B), P404 (F), P495 (K17), P497 (F27), P494 (K17), P496 (F27), P415 (G), P490 (T3), P428 (J), P405 (F), P436 (H1), P402 (F), P451 (E7), P487 (T3), P488 (T3), P489 (T3).

[b] All abbreviations as in the nomenclature section of the Results. Each mutant was tested against each of the bacteriophages used in this study, and every case of resistance is included in the table.

[c] Mutant class which is characteristically resistant to the bacteriophages.

bacteriophage specific to the group, and this after nitrosoguanidine mutagenesis. These mutants appear to be single mutants, since two have been mapped and the others do not have the combined properties of the other mutants (Hancock and Reeves, manuscript in preparation). They fall clearly into two subgroups (Table 8). Wrm mutants are resistant to bacteriophages from 8 out of the 11 other sets defined in this study and show resistance to between 30 and 33 bacteriophages. These mutants may be similar to other mutants described in the literature (15, 26).

DISCUSSION

In this study we selected bacteriophage-resistant mutants in *E. coli* K-12 using a wide range of different virulent bacteriophages. The results (Table 9) show that the 526 mutants fell into 11 main groups and 1 group of 5 miscellaneous mutants that did not fit well in any of the other groups. Each group of resistant mutants was characterized by a particular set of bacteriophages to which most of the mutants showed some degree of resistance. Although the mutants within a group were not always identical and a total of 30 different subgroups was noted, we had no difficulty in recognizing the relationship among them. A number of the mutant groups have already been described, including *ton* A, *ton* B, *bfe*, *tsx* and *con* (13, 16, 23, 30), the last having been isolated in this study. In addition to these well-defined mutant types, we describe a number of new groups of resistant mutants. However, we have found that extending the number of bacteriophages studies did not greatly increase the range of mutant types, suggesting that there are not many undiscovered types. It remains possible, of course, that other types remain undetected because they occur less frequently than those described previously or in this paper. However, the use of 62 bacteriophages has enabled us to define the cross-resistance patterns of all the mutants in some detail and attempt to arrange them in a meaningful way.

Each type of mutation must have its effect on bacteriophage resistance by preventing some stage of the bacteriophage infectious cycle. The best characterized mechanism is by loss of receptor, although not all mutants act at the level of adsorption. We have found many examples of receptor mutants in our studies (Hancock and Reeves, unpublished data); however, the poor adsorption, generally, of many of our bacteriophages, using a wide range of techniques, would make it difficult to identify tolerant mutants similar to those found in studies of colicin-resistant mutants (28). Garen and Kozloff (18) have postulated a class of resistant mutants with subtle surface modifications. They propose that the rate of phage attachment to these mutants would be slow, but still sufficient to prevent colony formation in the presence of bacteriophages. We have mutants that appear to be inhibited by certain bacteriophages, and it is possible that they may be of this class. Wahl (35) has described semiresistance which may be similar to our partial resistance with inhibition (IP) pattern of resistance. Carta and Bryson (14) have described mutants of *E. coli* B/r that are variably resistant to bacteriophage T1, in that greater than 20% of the mutants in a given population are in the sensitive state. This sensitive subpopulation does not consist of revertants. Bacteriophage T1 forms hazy plaques on the mutant strain due to this phenomenon and some of our mutants may be of the above type. One cannot rule out entirely phage tail-associated lysozyme as causing the inhibited type of resistance; however, the size of the inhibited plaques (comparable with the wild-type plaques) indicates that some bacteriophage propagation is necessary.

The various mutant groups we describe serve also to define sets of bacteriophages, most of which are unable to form plaques on more than one of the mutant groups described. Correspondingly, some of the mutant groups confer resistance to more than one set of bacteriophages. In general, the mutants seem to be of two main types. The majority of resistance groups confer resistance to one or two sets of bacteriophages, whereas the Bar and Wrm mutants are resistant to bacteriophages from many sets. Mutants of the first type are presumably affected in a cell component necessary for only one or two bacteriophage sets during infection, whereas the Bar and Wrm mutants are presumably affected in a function necessary, either directly or indirectly, for infection by bacteriophages of many different bacteriophage sets. One possibility (Hancock and Reeves, manuscript in preparation), is that Bar and Wrm mutants are affected in the cell surface in such a way that many different receptors are either absent or nonfunctional.

We find it interesting that the bacteriophages with B1-type morphology (1) that we studied, all resembled either T1, or T5 and BF23, in their dimensions. Most bacteriophages with similar dimensions to T1 resembled T1 in requiring both the *ton* A and *ton* B functions for infection; the others resembled T5 in requiring only *ton* A function, or BF23 in requiring only *bfe* function. It seems that many of the bacterio-

TABLE 8. *Mutants of the Wrm resistance group*

Subgroup	Mutants[a]	Resistance[b]																				
		K10	H3	H1	H8	K18	K31	Ox1	K9	T6	K3	K4	K5	Ac3	Ox3	M1	Ox2	Ox4	E4	K2	K20	K21	K29
1	P435			R	SL	R	SL		P					R	R	R	R	R	R	R	R	R	R
	P479			R	SL	R	P	SL						R	R	R	R	R	R	R	R	R	R
2	P416			R	R	SL	R	SL	R	SL	R	R	I	SL	SL	SL	R	R	R	SL	P	R	
	P424			R	R	SL	R	SL	R	SL	P	R	I	SL	SL	SL	R	R	R	SL	R	R	
	P239	I		R	P	SL	R	SL	R	SL	R	I	I	SL	SL	SL	R	R	R	R	P	P	R
		Ktn[c]		Tsx[c]							Con[c]								Efr[c]	Ktw[c]			

Subgroup	Mutants[a]	Resistance[b]																	
		Ox5	K16	F27	H^+	V	E7	K17	A	T3	T4	K19	T2	T7	E11	ϕ1	W31	K15	H
1	P435	R	R	R	R	R	R	P	R	R	SL	R		P		SL		R	SL
	P479	R	R	R	R	R	R	SL	R	R	SL	R		SL	SL	R	SL	R	SL
2	P416	R	R	R	R		R	SL	R	R	R		SL					R	P
	P424	R	R	R	R		R	SL	R	R	R		SL					P	
	P239	R	P	R	R		SL	SL	R	R	P		SL					R	P
		Ttk[c]							Bar[c]		Ttk[c]			Wrm[c]					

[a] The bacteriophages that the mutants were selected against were P416 (against C), P424 (T4), P435 (H1), P479 (T7), P239 (K29).

[b] All abbreviations as per nomenclature section of Results. Each mutant was tested against each of the bacteriophages used in this study and every case of resistance is included in the table.

[c] Mutant group which is characteristically resistant to the bacteriophages.

TABLE 9. *General pattern of resistance*

Resistance[a] group (subgroup)	Bacteriophages resistant to:[b]																														
	T5-like	T1-like	BF23-like	K10	H3	H1-like	T6	K3-like	Ac3-like	M1	Ox2-like	E4	K2	K20-like	K29	Ox5	K16	F27	H+	V	E7	K17	A	T3	T4	K19	T2	T7-like	K15	H		
TonA	R	R																													
TonB		R																													
Bfe			R																												
Ktn				R																											
Tsx (1)					R	R	R																								
(2)					R	V	V																								
Con								R	R	R	R					R															
Efr												R																			
Ktw (1)													V	V	V																
(2)												V	V	R	R																
(3)													V	V	V				V												
Ttk (1)												R				V	R	R	R	V						R	R				
(2)																V	R	R	R	V	R	V			R						
(3)																V	R	R	R	R	V				R	R					
(4)																V	R	R	R	R						R					
Misc. (1)																	V		R		V	V			V						
(2)																					V				V						
(3)																	V	R	V												
(4)																V	V	R	V												
(5)												V				V	V					V									
Bar (1)											V	V	V	V	V	V					V		V								
(2)										V	V	R	V	R	V	R	V	R		R		V									
(3)										V	V	R	V	V	V	R	V	V	V	V	V	V	V								
(4)										V	B	V		V	V	R	V	R	R		R		R	R							
(5)										V	V	R	R	V	V	R	V	R		R	R	R	V	V							
(6)										V	R	R	R	R	R	R	R	R	R		R		R	R	V						
(7)										V	V	R	V	V	V	R	V	R	R	V	R	V	R	R	V						
(8)										V	R	V	V	R	R	R	R	R	R	R	V	V	R	R	V	R					
Wrm (1)						V		R	R	R	R	R	R	R	R	R	R	R	R	R	R	V	R	R	V	R		V	R	V	
(2)				V		V	V	V	V	V	R	R	V	V	V	R	R	R	R		V	V	R	R	V		V		V	V	

[a] Subgroups are included in parentheses.

[b] When two or more bacteriophages have a similar pattern of resistant mutants, they have been grouped, and a representative bacteriophage has been nominated to describe this set of bacteriophages. T1-like, T5-like, and BF23-like: See legend Table 2. H1-like: H1, H8, K18, K31, Ox1, K9. K3-like: K3, K4, K5. Ac3-like: Ac3, Ox3. Ox2-like: Ox2, Ox4. K20-like: K20, K21. T7-like: T7, E11, ϕ1, W31. Resistance is described as either of the following. (R) Mutants in the group or subgroup are fully resistant to all the bacteriophages. (V) some of all of the mutants are not fully resistant and/or not all of the bacteriophages involved are able to lyse the mutant.

phages resembling T1 morphologically have similar requirements for infection, whereas those resembling T5 and BF23, probably all of one species, use either the T5 or BF23 receptor and, like the T1 set, are unaffected by the mutations of Bar or Wrm mutants.

Bacteriophages of A2 morphology are far more heterogeneous with respect to activity spectra on resistant mutants. Seven of our mutant groups are resistant to bacteriophages of this type. The mutants of three groups, Tsx, Con, and Ktw, are each resistant to a specific set of between four and eight bacteriophages with A2 morphology. Within the set of bacteriophages affected by the *tsx* mutations, H3 has only the one requirement, for the *tsx* function. This function is also required by T6 and six other bacteriophages of A2 morphology, which in addition have altered activities on one or both subgroups of the Wrm resistance group. Heterogeneity also exists among those bacteriophages that require the Con function. Only the Con mutants and one subgroup of Wrm mutants have altered sensitivity towards bacteriophage K3; however, in the case of bacteriophage Ox5 there are also mutants in the Ttk, Bar, and miscellaneous groups that are resistant to it.

Bacteriophage E4 of A1 morphology has a

complex set of requirements for infection judging by the variety of mutants resistant to it. Besides its specific resistant mutant, Efr, mutants from the Ttk, Ktw, Bar, Wrm, and miscellaneous groups are resistant to it, although in all except the Bar and Wrm groups these E4-resistant mutants are limited to one or two examples per group. It is not known whether these mutants each represent specific requirements for infection, or whether they demonstrate the general sensitivity of the bacteriophage E4 receptor to outer membrane alterations.

The activity spectra of 56 bacteriophages (represented in Table 9) on the resistant mutants are clearly very variable; however, we have been able to divide the bacteriophages into five main types: (i) those bacteriophages for which we have only one type of fully resistant mutant (including bacteriophage H3, the BF23-like bacteriophages, and the T5-like bacteriophages); (ii) those bacteriophages for which we have two types of fully resistant mutants, e.g., the T1-like bacteriophages; (iii) those bacteriophages for which we have two types of resistant mutants, one of which is specific and confers full resistance to a set of bacteriophages, whereas the other is cross-resistant to many bacteriophages. This second type of mutant is not fully resistant to all members of the bacteriophage set. The bacteriophages included in this category and their fully resistant mutants are T6, H1, H8, K9, K18, K31, Ox1 (Tsx), K3, K4, K5, Ox3, Ac3 (Con), K10 (Ktn), and T2 (Ttk). (iv) This type includes bacteriophages for which we have a range of mutants that are resistant, to varying degrees, to different sets of bacteriophages. Most of these bacteriophages are included in the Bar, Ktw, and Ttk sets (Tables 5 to 7). (v) This type includes bacteriophages K15 and H and the T7-like bacteriophages (Table 9 footnotes), which are unusual in that the only mutants (Wrm) found to be resistant to them in this survey are those cross-resistant to a wide range of bacteriophages, implying that for resistance to occur a large change in the cell surface must take place such that it interferes with the binding of many other bacteriophages to their receptors.

Resistance of a gram-negative cell to bacteriophages is mainly caused by changes in the outer membrane. The alterations lead to a variety of phenomena including colicin tolerance (28), loss of colicin receptor (28, 29), antibiotic supersusceptibility (34), alteration of the lipopolysaccharide (34), loss of a cell wall protein (9, 10, 27, 29), and alteration in requirement for an amino acid (3). A study of these pleiotropic effects of mutations to bacteriophage resistance has led to a better understanding of the structure and function of the cell surface. In this study we have extended the variety of bacteriophage-resistant mutants known and have classified them. Studies on one of the new mutant types (Con^-) has already led to research on conjugation (30), and we hope that further study of the other mutants will lead to a better understanding, not only of bacteriophage infection, but of other surface-related phenomena. Preliminary studies of the properties of these mutants will be presented (Hancock and Reeves, manuscript in preparation).

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