Genet. Res., Camb. (1966), **7**, *pp.* 223–234
Printed in Great Britain

Stable and unstable alterations of the host-induced modification properties of *Escherichia coli* B, K and C

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(*Received* 8 *April* 1965)

1. INTRODUCTION

The system of host-induced modification (HIM) in *E. coli* B, K and C (Arber & Dussoix, 1962) has the following properties. Normal K, which is able to restrict phage λ grown in either B or C and confers a K-specific modification to λ-DNA synthesized in it, can be represented as $K\ r^+m^+$. Similarly, normal B can be represented as $B\ r^+m^+$, indicating that it restricts phage λ grown in either K or C and that it confers a B-specific modification to λ-DNA synthesized in it. Strain C accepts phage λ grown in K, B or C without restriction. Recently the genes controlling restriction and modification have been mapped close to the marker threonine and on the opposite side of it to leucine (Colson, Glover, Symonds & Stacey, 1965). In the course of this work, strains were isolated which had HIM properties unlike those of B, K or C.

These strains display different patterns of HIM. One such pattern can be represented as $B\ r^-m^+$, indicating that the ability to restrict λ grown in K or C has been lost while the ability to confer the B-specific modification is retained. Another can be represented as K r^-m^- or B r^-m^-: these strains have lost the ability to restrict λ.C and to confer respectively either the K- or the B-specific modification. Others are more complex and comprise strains which on first isolation appear to have HIM properties intermediate between those of B, K and C, but which on further analysis turn out to be unstable. We shall describe the isolation of these strains and present some details of their behaviour.

2. MATERIALS AND METHODS

The media, bacterial strains and techniques used are all described by Colson *et al.* (1965).

3. RESULTS

(i) *Experiments with F*-lac *infected strains*

The episome F-*lac* was transferred by conjugation from a K F-*lac*$^+$ donor to *lac*$^-$ mutants of B and C, and the B F-*lac*$^+$ and C F-*lac*$^+$ strains obtained were then used as donors to transfer F-*lac* to each of the strains B, K and C. The relative

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efficiencies with which F-*lac* is accepted by the strains are shown in Table 1, together with the plating efficiencies of λ.B, λ.K and λ.C for comparison. It is clear that F-*lac* undergoes the same pattern of HIM as does phage λ (Arber & Dussoix, 1962) but that the level of restriction is very much lower. In some experiments C turns out to be a better acceptor of F-*lac* than the homologous strain.

Table 1. *The relative efficiencies of acceptance of F*-lac$^+$ *and λ by* E. coli *B, K and C*

lac$^-$ recipient	Donor F-*lac*$^+$ B	F-*lac*$^+$ K	F-*lac*$^+$ C
B*	1·0	0·3	0·1
	(1·0)	(10^{-4})	(10^{-4})
K*	0·5	1·0	0·2
	(10^{-4})	(1·0)	(10^{-4})
C*	1·0	2·0	1·0
	(1·0)	(1·0)	(1·0)

In each experiment the same culture of the F-*lac*$^+$ donor was used as a minority parent to infect each of the three recipients B, K and C. The number of *lac*$^+$ infected bacteria was scored on media selective for the recipient.

The figures in brackets are the approximate efficiencies of plating of phage λ.B, λ.K and λ.C** grown on the donor bacteria B, K and C respectively and plated on the recipients.

* B = *E. coli* B251 (Arber & Dussoix, 1962); K = *E. coli* K12; C = *E. coli* C (Bertani & Weigle, 1953).

** Following the notation of Arber & Dussoix (1962) the host specificity of a phage is represented by the name of the phage followed by the name of the host strain in which it was last grown. For example, λ.C means phage lambda which has been grown in *E. coli* C.

In spite of the fact that the level of restriction was only 10-fold there was reason to suppose that the bacteria infected with F-*lac* would be enriched for the proportion of non-restricting mutants (Glover *et al.*, 1963). Therefore colonies of B, K and C which had been infected with F-*lac* from a different strain were purified and tested with stock suspensions of λ.B, λ.K and λ.C for restriction and modification. Only 11 out of 730 colonies tested had altered properties of HIM, and these are listed in Table 2. Two colonies of B infected with F-*lac* from K did not restrict λ.B, λ.K or λ.C, but phage λ grown in them carried the normal host-specificity of B. These are designated B r^-m^+ and were pure when first isolated on selective media. The remaining nine colonies did not score as either r^+ or r^- for restriction, nor as m^+ or m^- for modification. Each of them was analysed by repeated single colony isolation on selective media and the results of this clonal analysis are summarized in Table 3.

The unusual feature of these experiments is that most of the strains with altered HIM properties are unstable on first isolation. During subsequent single colony purifications they can segregate three types of progeny: one which has the donor pattern of HIM, one which has the HIM properties of the recipient and one which is unstable. In those clones analysed after four single colony purifications this unstable trait had disappeared.

Three different kinds of unstable HIM have been observed. For unstable clones 3, 4, 5 and 8 (Table 3) the observation is that cultures grown from colonies on the

first purification plates accept λ.B and λ.K equally well but restrict λ.C, and the λ phage from plaques on these bacteria plates equally well on K and B indicator bacteria. For unstable clone 10 the observation is that λ.B, λ.K and λ.C plate with an efficiency of one on this strain, but λ phage from plaques on these bacteria plates with a higher efficiency on B than on K indicator bacteria. For unstable clone 12 the observation is that λ.B, λ.K and λ.C are not restricted, but λ phage from plaques on this strain plates with a higher efficiency on K than on B indicator bacteria.

Table 2. *Changes in HIM among* E. coli *B, K and C infected with* F-lac

Expt. No.	F-*lac*$^+$ donor	*lac*$^-$ recipient	Number of F-*lac*$^+$ infected recipients tested	Number of F-*lac*$^+$ infected recipients with altered HIM properties	New HIM phenotype
1	K *met*$^-$	B prototroph	20	2	B r^-m^+
2	K *met*$^-$	B *cys*$^-$	36	0	
3	K *met*$^-$	B *isol*$^-$	100	1	unstable
4	K *met*$^-$	B *isol*$^-$	45	1	unstable
5	K *met*$^-$	B *try*$^-$	120	3	unstable
6	K *met*$^-$	C prototroph	8	0	
7	K *met*$^-$	C *try*$^-$	19	0	
8	B *isol*$^-$	K *thr*$^-$*leu*$^-$*thi*$^-$	100	2	unstable
9	B *isol*$^-$	C *try*$^-$	54	0	
10	C *his*$^-$	B *cys*$^-$	12	1	unstable
11	C *his*$^-$	B *try*$^-$	100	0	
12	C *his*$^-$	K *thr*$^-$*leu*$^-$*thi*$^-$	116	1	unstable

cys = cysteine, *his* = histidine, *isol* = isoleucine, *leu* = leucine, *met* = methionine, *thi* = thiamin, *thr* = threonine, *try* = tryptophane.

This behaviour is what can be expected from mixtures of strains B plus K, C plus B or C plus K. In fact in reconstruction experiments roughly equal numbers of the two components used as indicator bacteria show precisely this result. For example λ.B, λ.K and λ.C plate with equal efficiencies on a 1:1 mixture of B and C indicator bacteria and λ phage from plaques grown on this mixture plates with an efficiency of about 10^{-4} on K and with an efficiency of about one on B or C. The segregation patterns we have found confirm that the unstable clones do indeed contain two components.

It is interesting to compare the results in Table 3 with those of Glover, Schell, Symonds & Stacey (1963), who carried out a series of precisely analogous experiments with F-*lac* between K and K(P1) in which the HIM of phage λ is controlled by the prophage P1 (Arber & Dussoix, 1962). They found that F-*lac* was restricted by P1 100-fold and about 33% of the K(P1) bacteria which accepted the F-*lac* contained P1 with altered properties of HIM. Restriction of F-*lac* between B, K and C is in contrast only about 10-fold, and the frequency of strains with altered properties of HIM among recipients infected with F-*lac* is much rarer.

Table 3. *Types of HIM among unstable colonies of* E. coli *B, K and C* infected with *F-lac*

Expt. No. from Table 2	Direction of F-*lac* transfer	Number of colonies with HIM phenotype indicated after serial single colony purification			
		1st purification	2nd purification	3rd purification	4th purification
3	K→B	unstable	3 K		
			7 B		
			1 unstable	7 K	
				2 B	
				1 unstable	
4	K→B	unstable	4 B		
			1 unstable	3 K	
				1 unstable	
				1 unstable	
5	K→B	unstable	9 K		
			1 unstable	10 K	
		unstable	9 B		
			1 unstable		
		unstable			
8	B→K	unstable	2 B		
			1 unstable	10 B	
			1 unstable	9 B	
				1 unstable	10 B
			1 unstable	1 K	10 K
				1 B	10 B
				1 B	10 B
				6 B	
				1 unstable	
		unstable	8 K		
10	C→B	1 unstable	10 B r^-m^-		
12	C→K	1 unstable	14 K r^-m^-		
			1 unstable	8 K	
				1 K r^-m^-	10 K r^-m^-
				1 unstable	10 K

Colonies isolated on selective media were restreaked on the same medium (1st purification) and then further purified by serial single colony isolations on the same medium (2nd 3rd and 4th purification). In some experiments the 1st purification was also to single colonies.

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De Haan *et al.* (1963) found that the transfer of F-*lac* from K to B was very inefficient (8×10^{-5}), and that the majority of the *lac*$^+$ colonies isolated had the *lac*$^+$ marker stably integrated on the chromosome and were F$^-$. In our experience the *lac*$^+$ colonies obtained by F-*lac* infection from K to B are good donors of F-*lac* when tested for F-*lac* transfer back to K. They can effect chromosomal transfer from B to K, and they lose the donor capacity and the *lac*$^+$ character together when cured with acridine orange. These differences are due to differences in the B strains which we have each used as recipients. We used B251 (Arber & Dussoix, 1962), and de Haan *et al.* (1963) used another B strain CB90 (Rörsch, 1963).

(ii) *Induction of HIM mutants of K by nitrosoguanidine*

A suspension of K containing 10^9 bacteria per ml. in buffer was treated with nitrosoguanidine for 3½ hours at 37°. The treated bacteria were then subjected to

Table 4. *The isolation of K* r$^-$m$^-$ *from unstable clones of* E. coli *K* r$^+$m$^+$ *after nitrosoguanidine treatment and indirect selection for decreased ability to restrict λ. B and λ. C*

Culture number	Number of colonies with HIM phenotype indicated after serial single colony purification		
	1st purification	2nd purification	3rd purification
1	10 K r^+m^+		
2	7 K r^+m^+		
	2 K r^-m^-		
	1 unstable	10 K r^+m^+	
3	9 K r^+m^+		
	1 K r^-m^-	10 K r^-m^-	
4	6 K r^+m^+		
	4 K r^-m^-		
5	8 K r^+m^+		
	1 unstable	6 K r^+m^+	
		1 K r^+m^+	10 K r^+m^+
		1 K r^-m^-	10 K r^-m^-
		1 K r^-m^-	10 K r^-m^-
		1 K r^-m^-	10 K r^-m^-
	1 unstable	7 K r^+m^+	
		1 K r^+m^+	10 K r^+m^+
		1 K r^+m^+	10 K r^+m^+
		1 unstable	5 K r^+m^+
			5 K r^-m^-

https://doi.org/10.1017/S0016672300009630 Published online by Cambridge University Press

indirect selection (Cavalli & Lederberg, 1956) for increased ability to plate λ.B and λ.C. On the assumption that at least 1 in 10^4 of the treated bacteria would be a mutant, a series of 1 ml. cultures were grown from inocula containing approximately 10^3 treated cells. After over-night incubation an aliquot of each culture was tested for its ability to plate λ.B and λ.C. From the remainder of that culture which plated λ.B and λ.C at the highest efficiency, inocula were prepared containing approximately 100 cells. A series of 1 ml. cultures were grown from the inocula and the test repeated. At the end of three such enrichment cycles seven out of twenty cultures showed only a 10^{-1} restriction of λ.B and λ.C. Five of these seven cultures were plated out for single colonies and ten colonies from each were tested for their ability to restrict and modify phage λ. The results of these tests are summarized in Table 4. Cultures 2 and 5 produced clones of K r^+m^+, K r^-m^- and unstable clones. Cultures grown from these unstable clones do not resrtict λ.C or λ.K but λ phage picked from plaques on them appears to plate as well on K as it does on C. The unstable clones from culture 5 segregated K r^+m^+, K r^-m^- and unstable clones. This instability disappeared after three purifications.

These unstable clones originated from small inocula of nitrosoguanidine-treated K bacteria, which had been serially sub-cultured three times during the selection procedure and so were descended from the treated cells by many generations. This makes it extremely unlikely that the segregation pattern observed is due to the segregation of mutant and non-mutant nuclei from multi-nucleate cells. It is clear that culture 5 contained cells which have the potentiality to segregate K r^+m^+ and K r^-m^- bacteria and can multiply in this state.

(iii) *Unstable clones among transduced cells*

The loci controlling HIM in B and C can be co-transduced with the marker *thr*$^+$ by phage P1 (Colson *et al.* 1965). The transduced colonies exhibit the HIM properties of the donor but when first isolated some of them are unstable and segregate both donor and recipient types of HIM. An analysis of the segregation pattern in three such clones is summarized in Table 5. The pattern is similar to that of unstable clones isolated after F-*lac* infection or after nitrosoguanidine treatment.

However, one clone obtained by transduction of K r^-m^- with P1 phage grown on K r^+m^+ did not inherit the *r* gene from K, but did apparently inherit the *m* gene. This clone has been tested after each of nine serial single colony purifications and the results are presented in Table 6.

It is clear that none of the segregants is able to restrict λ.C. From the plating efficiency on K and C indicator bacteria of λ picked from plaques on them it would appear that a varying amount of the phage is host-modified for K. This can be accounted for in one of two ways: either the clone segregates a small fraction of cells in which the *m* gene is present and all of the phage produced by such cells are λ.K, or it segregates cells which produce mixed bursts of λ.K and λ.C. At the sixth and the ninth single-colony purifications half of each of ten colonies was used for the usual restriction and modification tests with phage λ. The remainder of the

colony which yielded the highest proportion of λ.K was used in a single burst experiment. This half colony was resuspended in 1 ml. of M/100 $MgSO_4$ and starved for ½ hour at 37°. The cells were infected with a multiplicity of ten λ.C particles and unadsorbed phage destroyed with antiserum. The infected bacteria were diluted in tryptone into a large number of tubes so that approximately one in ten tubes contained a single infected bacterium. The tubes were incubated for 90 min.

Table 5. *Segregation of different HIM phenotypes from unstable clones of* E. coli *B, K and C after transduction by phage P1 of the marker* thr+

HIM phenotype of donor	HIM phenotype of recipient	Number of colonies with HIM phenotype indicated after serial single colony purification		
		1st purification	2nd purification	3rd purification
B r^-m^+	B r^+m^+	unstable	5 B r^-m^+	10 B r^-m^+
			15 B r^+m^+	
K r^+m^+	K r^-m^-	2 K r^+m^+		
		1 unstable	6 K r^+m^+	
			4 K r^-m^-	
		1 unstable	9 K r^-m^-	For further details see Table 6
			1 unstable	
K r^+m^+	C	1 K r^+m^+		
		1 unstable	9 C	
			1 K r^+m^+	

at 37°, and then half the contents plated on K and the other half on C. Table 7 shows the results of these two single burst experiments. A total of 165 tubes gave nineteen bursts of which thirteen were pure λ.C and six contained a small number of λ.K. The colonies selected for these single burst experiments were those which, when infected with λ.C, produced a high proportion of λ.K yet no single bursts of pure λ.K were obtained. It is evident therefore that none of the segregants are stable K r^-m^+ though from the evidence of single bursts many would appear to be K r^-m^-.

(iv) *Unstable clones among recombinants from bacterial crosses*

The loci controlling HIM can be transferred in crosses from Hfr K r^+m^+ strains to K r^-m^- F^- recipients and to B F^- and C F^- strains (Colson *et al.*, 1965). In every cross in which there was inheritance of the HIM genes from the donor some of the recombinant colonies showed HIM properties characteristic of mixtures of donor and recipient bacteria. The frequency of such colonies varied from cross to cross and also varied from one recombinant class to another. In every cross there was

Table 6. *Clonal analysis of the HIM behaviour of the progeny from a colony of K* r⁻m⁻thr⁻ *transduced by a P1 lysate of K* r⁺m⁺thr⁺ *and selected for* thr⁺

Numbers of colonies with HIM behaviour of the type indicated after serial single colony isolation

1st isolation	2nd isolation	3rd isolation	4th isolation	5th isolation	6th isolation	7th isolation	8th isolation	9th isolation
***1 K $r^-m^\pm$	9 K r^-m^-							
		8 K r^-m^-						
			4 K r^-m^-					
			4 K $r^-m^\pm$					
			1 K $r^-m^\pm$	5 K r^-m^-				
				4 K $r^-m^\pm$				
		1 K $r^-m^\pm$		1 K $r^-m^\pm$	3 K r^-m^-			
					7 K $r^-m^\pm$			
			1 K $r^-m^\pm$	2 K r^-m^-				
				7 K $r^-m^\pm$				
				1 K $r^-m^\pm$	3 K r^-m^-			
	1 K $r^-m^\pm$				7 K $r^-m^\pm$			

		1 K $r^-m^\pm$	5 K r^-m^-				
			3 K $r^-m^\pm$				
			1 K $r^-m^\pm$	2 K r^-m^-			
				7 K $r^-m^\pm$			
				1 K $r^-m^\pm$	4 K r^-m^-		
					5 K $r^-m^\pm$		
					*1 K $r^-m^\pm$	2 K r^-m^-	
						8 K $r^-m^\pm$	
			1 K $r^-m^\pm$	1 K r^-m^-			
				8 K $r^-m^\pm$			
				1 K $r^-m^\pm$	3 K r^-m^-		
					6 K $r^-m^\pm$		
					1 K $r^-m^\pm$	3 K r^-m^-	
						6 K $r^-m^\pm$	
						1 K $r^-m^\pm$	5 K r^-m^-
							4 K $r^-m^\pm$
						1 K $r^-m^\pm$	5 K r^-m^-
							4 K $r^-m^\pm$
							**1 K $r^-m^\pm$

* Colony used in single burst experiment I, Table 7.
** Colony used in single burst experiment II, Table 7.
*** The symbol $m^\pm$ indicates that phage λ grown on the strain plates with an efficiency of 1·0 on C and between 1·0 and 10^{-3} on K.

Table 7. *Single burst experiments of phage λ.C grown in two unstable K* r⁻m± *strains*

Expt. No.	Total number of tubes	Number of tubes with a burst of λ	Number of plaques	
			On C	On K
I	75	7	53	0
			72	0
			1400	0
			81	1
			75	11
			361	0
			133	7
II	90	12	55	0
			166	0
			126	0
			79	0
			66	0
			33	0
			105	6
			99	0
			117	13
			228	1
			14	0
			96	0

The K $r^{-}m^{\pm}$ bacteria were starved for ½ hour at 37° in 0·01 M $MgSO_4$ and infected at a multiplicity of 10 with λ.C. Unadsorbed phage was destroyed by antiserum. After dilution, a large number of tubes were inoculated such that on average one in ten tubes contained a single infected cell. The tubes were incubated for 90 min. at 37° and half the contents plated on K and the other half on C.

selection against the genotype of the donor; so it is unlikely that these colonies are in fact simple mixtures of donor and recipient bacteria, but rather they are clones with the potentiality to segregate two patterns of HIM among their progeny. Clonal analysis of these colonies has not been carried out; but clone 8 in Table 3 is a genetic recombinant arising presumably as a result of chromosomal transfer from the F′ donor B to the F⁻ recipient K, and it shows all the features characteristic of the unstable clones we have analysed in the other experiments reported above.

4. DISCUSSION

In experiments designed to isolate strains with altered properties of HIM, some variants were obtained which were stable on first isolation. But the vast majority of them are unstable at first and segregate during many generations two different stable HIM phenotypes and a third which continues to segregate. Generally this third class eventually disappears after repeated serial single colony isolations. Since the potentiality to segregate frequently persists through two or more successive single colony isolations we have to rule out nuclear segregation and the persistence of donor bacteria as explanations of this segregation pattern.

It may be that a state of partial diploidy (Lederberg, 1949; Anderson, 1958; Lennox, 1955; Curtiss, 1964) exists in these strains, but only in one case is there evidence that a diploid fragment extending to markers other than those controlling HIM was present. It is certainly unlikely that a partial diploid is formed in the unstable clones derived from nitrosoguanidine-treated cells, yet similar segregation patterns were observed.

The most interesting unstable clone is that illustrated in Table 6. It was derived from a colony of K r^-m^- which had been transduced with a phage P1 lysate of K r^+m^+. The progenitor of the clones seems not to have inherited r^+ because all of the progeny are r^-. However, m^+ was apparently inherited since some at least of the progeny have the capacity to produce some λ.K. But the amount of λ.K produced is always less than would be obtained from a normal culture of K. This could be due to the presence in the culture of some cells in which m^+ is present and others in which it is not. This is apparently true because stable K r^-m^- segregants have been repeatedly isolated from the unstable clone. But the situation is not as simple as this. Single burst experiments have shown that in a culture which produces a high proportion of λ.K there are bacteria which produce bursts of pure λ.C, bacteria which produce both λ.K and λ.C but none which produce λ.K alone. Consistent with this is the fact that after nine single colony isolations no stable K r^-m^+ strain was obtained. If, as seems likely, there are more than two genes controlling HIM then these cells appear to have inherited only one of them, m^+. They appear to carry both the m^+ and the m^- alleles but the m^+ allele is not fully expressed. The expression of m^+ may depend on the presence of r^+, though the isolation of stable B r^-m^+strains makes this unlikely. Alternatively, the expression of m^+ may depend on the presence of another gene linked to r^+ but not inherited in this clone.

In a pedigree analysis by micromanipulation of the progeny of single zygotes from Hfr × F^- crosses, Anderson (1958) found that recombinants segregated late, between the third and ninth generations, and more than one recombinant type was found among the progeny of a single zygote. This result can be explained if the donor fragment persists, and either fails to multiply as in abortive transduction but nevertheless participates in a sequence of recombination events, or if it forms a partial but unstable diploid which segregates after a few generations. In crosses using a particular mutant, Lederberg (1949) and more recently Curtiss (1964) found that an appreciable portion of zygotes were diploid and when plated on non-selective media segregated both parental types as well as recombinants even after many single colony isolations. Lennox (1955) found that most colonies arising from transduced bacteria on selective media were mixed with regard to transduced markers and were still impure after up to five single colony purifications (about 150 generations). It is probable that the clones with unstable properties of HIM which we have observed after transduction and conjugation are also due to the persistence of donor fragments in partial diploids of this type. However, the unstable clones derived from nitrosoguanidine-treated cells are certainly unlikely to contain diploid fragments.

SUMMARY

Strains with altered properties of host-induced modification (HIM) have been isolated. Some of these variants were stable on first isolation but the majority of them are unstable. They segregate during many generations two different stable HIM phenotypes and a third type which continues to segregate. Usually this instability disappears during repeated serial single colony isolation, but in one case it still persisted after nine such single colony isolation steps. Evidence suggesting that they are unstable partial diploids is discussed.

We wish to thank Mr N. Reed for efficient technical assistance and one of us (C. C.) is grateful to the British Council for a grant during the latter part of this work.

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