SHORT PAPERS

Mechanically caused damage to Hfr cells of *Escherichia coli* K12

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INTRODUCTION

Brinton, Gemski & Carnahan (1964) have described 'sex-pili' specifically associated with the donor condition in *Escherichia coli* K12. Without providing data they mentioned the feasibility of removing the sex-pili from the surface of donor cells and regeneration of these organelles. This paper describes experiments of a similar kind in which donor cells were damaged by treating either mating pairs or unmated donor cells in a Waring Blendor or similar device. The damaged donor cells were unable to form zygotes and unable to adsorb the male-specific coli-phage $\mu2$ (Dettori, Maccacaro & Piccinin, 1961). The damage is only temporary, being repaired by growth unless chloramphenicol is present. The damage reported here differs from the lethal effects reported by Clowes (1963) and Gross (1963) who described cellular damage associated with mating in *E. coli* K12. They observed death of F⁻ cells during conjugation when the ratio of Hfr to F⁻ organisms is raised above about 0·2 (Gross, 1963).

MATERIALS AND METHODS

Bacteria were grown in M9 medium (see Adams, 1959, p. 446) supplemented with vitamin-free Difco casamino acids (0·2% w/v) and vitamin B_1 to a final concentration of 5 μg./ml. 0·2% w/v D-glucose was provided as carbon source and 5 μg./ml. of thymine was provided for thymine auxotrophs. This medium is referred to as 'complete M9'.

Dilutions of the mating mixtures were spread on selective plates (Fisher, 1957) and thr⁺leu⁺ recombinant colonies enumerated after 24 hours incubation at 37°. Viable counts were determined by plating dilutions on nutrient agar (Oxoid No. 2, solidified with 1·25% w/v Davis New Zealand agar) supplemented with thymine (20 μg./ml.). Total cell counts were performed with the aid of a Coulter Counter Model A, fitted with a 30 μ aperture.

Donor cell suspensions were adjusted to the same density (approx. 10^8 bacteria/ml.) and exposed to coliphage $\mu2$ at a multiplicity of 10 plaque-forming units. After 7 min. at 37°, adsorption was terminated by the addition of anti-μ antiserum. Dilutions were plated on strain 89 and plaques counted after overnight incubation at 37°. Both the coliphage $\mu2$ and the anti-μ antiserum were kindly donated by Dr Elinor Meynell. All of the bacterial strains employed are derivatives of *Escherichia coli* K12. Strains AB250, AB259 and AB2383 were provided by Dr E. A. Adelberg.

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Hfr strains: 89 ($thy^-B_1^- lac^-$) is a thymine-requiring derivative of AB259 (Bouck & Adelberg, 1963), obtained by the method of Okada, Yanagisawa & Ryan (1960) and will be fully described elsewhere. 131C2 ($met^-thy^-ileu^-$) is an isoleucine-requiring derivative of AB2383 isolated after treatment of the parent strain with N-methyl-N'-nitro-N-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.).

F$^-$ strains: 94 is AB250 ($thr^-leu^-his^-B_1^-$). 132 ($thr^-leu^-his^-B_1^-str\text{-}r$) was derived from AB250 by selection for resistance to 250 μg/ml. streptomycin. Relative positions of some markers, origins and directions of transfer of Hfr strains are given in Fig. 1.
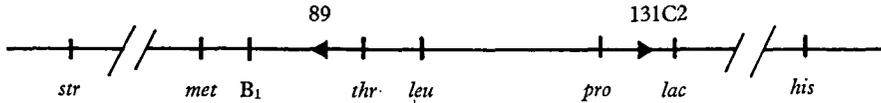

Fig. 1. A portion of the linkage group of *Escherichia coli* K 12 showing approximate positions of some loci involved in the crosses employed. The arrow heads indicate the origin and direction of transfer by the two Hfr strains employed, 89 Hfr and 131C2 Hfr.

RESULTS

Damage to ex-conjugant Hfr cells

Logarithmically growing cells of 131C2 Hfr, 94 F$^-$ and 132 F$^-$ were harvested, washed on membrane filters (Schleicher & Schüll, type B6 unbacked) and resuspended in fresh, warm, complete M9. Total counts were determined and the suspensions of 131C2 Hfr and 94 F$^-$ (*str-s*) mixed at 37°. After 30 min., mating pairs were separated by vigorous agitation in either a Waring Blendor or M.S.E. Homogenizer. A sample of the mixture was diluted and plated for thr$^+$leu$^+$ recombinants on selective agar. At the same time strain 132 F$^-$ (*str-r*) was added to the mixture and incubation continued at 37° for a further 30 min. when a second sample was blended, diluted and plated on selective agar supplemented with streptomycin. Only thr$^+$leu$^+$ recombinants inheriting the *str-r* locus from F$^-$ 132 can grow on these plates. The results of such an experiment are given in Table 1.

Table 1. *Ability of Hfr 131C2 to form zygotes in successive matings with F$^-$ strains*

Dilution of mating mixture	Recombinants per 0·1 ml. plated	
	1st mating	2nd mating
10^{-3}	135	0
10^{-2}	Not countable	6
10^{-1}	Not countable	51

The table shows the average number of thr$^+$leu$^+$ recombinants per 0·1 ml. of diluted mating mixture, issuing from successive matings of Hfr 131C2 with a *str-s* F$^-$ (94) and a derivative *str-r* F$^-$ (132). Mating mixtures were prepared in complete M9 from 1 ml. Hfr 131C2 ($2\cdot1 \times 10^8$/ml.) and 9 ml. of *str-s* F$^-$ ($2\cdot3 \cdot 10^8$/ml.). After 30 min. at 37° the mixture was blended for 30 sec., a sample was removed for dilution and plating on minimal agar supplemented with vitamin B$_1$ and histidine. At the same time 9 ml. of *str-r* F$^-$ ($2\cdot25 \times 10^8$/ml.) was added to the mixture and mating continued for a further 30 min. Another sample was blended, diluted and plated on minimal agar supplemented with histidine, vitamin B$_1$ and streptomycin.

Although fewer recombinants would be expected from the second mating due to dilution of the mixture and to competition between the *str-s* and *str-r* F$^-$ strains, the number observed is less than 5% of that expected, assuming that the Hfr strain produces thr$^+$leu$^+$ recombinants equally with both F$^-$ strains. Control matings showed that 132 F$^-$ (*str-r*) produced at least as many recombinants as 94 F$^-$ (*str-s*) in conjunction with 131C2 Hfr. Since the second mating produced fewer recombinants than expected it was concluded that the Hfr cells were damaged in some way which prevented their participating in second matings.

Mechanical emasculation of Hfr organisms

Exponentially growing cells of strain 89 Hfr, suspended in complete M9, were agitated vigorously in an M.S.E. Homogenizer and assayed before and after treatment for viable count, ability to form zygotes and ability to adsorb the male specific phage $\mu2$ (Dettori *et al.*, 1961). The results of these experiments are shown in Tables 2 and 3. Treatment in

Table 2. *Mechanical infliction and repair of damage to zygote-forming ability*

Treatment of Hfr cells	Hfr cells/ml. ($\times 10^{-8}$)		Number of recombinants	
	Total count	Viable count	Per ml. $\times 10^{-6}$	Per 10^4 Hfr
Before blending		1·9	3·94	210
After blending	2.3	2·0	0·32	16
After blending and 60 min. incubation in complete M9 + 20 μg./ml. chloramphenicol	3·6		0·13	3·5
After blending and 60 min. incubation in complete M9	5·4		2·55	48

Cultures of 89 Hfr and 132 F$^-$ were grown in complete M9 to c. 2×10^8 organisms per ml., at which point 132 was placed in an ice bath. 10 ml. of culture 89 were treated with the M.S.E. homogenizer for 3 min. and viable counts determined on the treated and untreated suspensions. Two mixtures were prepared (0 min.) containing 1 ml. of 132 and 0·1 ml. of the untreated and treated suspension of 89, respectively, and were placed at 37° for 5 min. They were then diluted 10^{-2} in M9 + aspartic acid + thymine + B$_1$ (see Fisher, 1957, regarding aspartic acid) at 37° and left for a further 25 min. Pulse mating technique was employed to reduce errors due to recovery of mating ability which might occur during the time allowed for transfer of genetic material. Duplicates of suitable dilutions were plated on minimal agar supplemented with histidine, B$_1$, and streptomycin.

Also at 0 min. the remainder of culture 89 was divided into two aliquots, *a* and *b*. Both were incubated at 37° for 60 min. but *b* was incubated in presence of 20 μg./ml. of chloramphenicol. At 60 min. twin mixtures of 1·0 ml. 132 and 0·1 ml. of (*a*) and (*b*) respectively, were prepared. These mixtures were incubated and diluted as in the first part of the experiment and plated on minimal agar supplemented with histidine, B$_1$ and streptomycin.

the homogenizer does not significantly affect the viable count but it has a pronounced inhibitory effect on both the adsorption of $\mu2$ and the ability of Hfr organisms to form zygotes. Both forms of damage can be repaired by growth of the Hfr organisms in complete M9 unless inhibitory concentrations of chloramphenical are present. Under the latter conditions there is little recovery of either the ability to adsorb coliphage $\mu2$ or the

Table 3. *Mechanical infliction and repair of damage affecting coliphage μ2 adsorption by strain* 89

	Hfr cells/ml. $\times 10^{-8}$		Infective centres per Hfr $\times 10^3$	
	− CM	+ CM	− CM	+ CM
Before blending	2.69*		14	
Time of incubation after blending				
0 min.	2·05*		4·9	
20 min.	2·35	2·31	4·5	3·9
40 min.	3·26	2·75	6·4	4·7
60 min.	4·06	2·70	9·4	3·0

* Viable count.

Strain 89 was grown in complete M9 to c. 2×10^8 organisms/ml. An aliquot was removed for viable count determination and to determine the adsorption of coliphage μ2. The remainder was treated for 3 min. in the M.S.E. Homogenizer. A second aliquot was then removed for determination of viable count and ability to adsorb coliphage μ2. The remainder was divided equally and chloramphenicol (CM) (50 μg./ml. final concentration) added to one half. Both cultures were reincubated at 37° with shaking. Samples were removed at 20, 40 and 60 min., filtered, washed and resuspended at a cell density of c. 10^8/ml. in complete M9. Total counts were performed before and after washing and resuspension and the ability of the resuspensions to adsorb μ2 was determined.

ability to form zygotes. Even in the absence of chloramphenicol neither of these properties is restored to the original level after 1 hour. The reason for this may be that cell densities are such that there is a substantial number of non-dividing cells present. Evidence that exponentially growing cells have maximum fertility has been presented (Fisher, 1965).

DISCUSSION

Several authors have shown that donor cells of *Escherichia coli* K12 are endowed with specific cellular organelles, sex-pili (Brinton *et al.*, 1964; Valentine & Strand, 1965). The experiments reported here confirm the preliminary report of Brinton *et al.*, 1964, since it can be inferred that the pili can be damaged by blending. The inability of damaged cells to form zygotes could be attributed either to (1) a lack of intact pili or (2) to the possibility that donor cells whose chromosome was severed in course of transfer cannot reinitiate transfer until replication of the chromosome has recycled to a specific point. There are indications that the second interpretation is not correct, viz. (1) we would not expect treatment of the unmated cells in the homogenizer would prevent their mating and (2) the fact that recovery from damage to μ2-adsorbing capacity closely parallels recovery from damage to zygote-forming ability. It is also known from the work of Brinton *et al.* (1964) that male-specific phages adsorb exclusively to the sex-pili.

The lack of recovery of maleness in the presence of chloramphenicol (Tables 2 and 3) is indicative of a requirement for protein synthesis. Because the lack of recovery is total, it seems likely that the chloramphenicol is acting directly against the synthesis of a protein component of the sex-pili rather than against the synthesis of an enzyme involved in their synthesis. However, in the absence of better quantitative data one cannot be too dogmatic on this point.

The experiments do not distinguish between the possible roles which can be suggested for the sex-pili, viz. of acting as a 'grappling hook' to facilitate pair formation between donor and recipient cells or of acting as a conjugation tube to channel the DNA from the donor to the recipient cell.

SUMMARY

When mating pairs of *Escherichia coli* K12 are separated by physical means, e.g. by violent agitation, the Hfr cell is unable to mate with a second F⁻ cell. This effect can be simulated by agitation of the Hfr cells prior to mating and, in addition, such cells can be shown to have an impaired ability to adsorb the male-specific coliphage $\mu 2$. Inhibitory concentrations of chloramphenicol block the repair of the damage.

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