Colicin factors and Episomes

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Colicins are protein-like antibiotics, produced by certain strains of *Enterobacter*, which are lethal for many other strains of *Enterobacter* (Fredericq, 1953). The ability of a bacterial strain to produce colicin (colicinogeny) is a stable and heritable character, and is presumed to be due to genetic determinants termed colicin factors (*C*-factors). Many types of *C*-factor exist, determining colicins of various kinds; they can be transferred from one colicinogenic strain (*col*$^+$) of *Enterobacter* to another, previously non-colicinogenic (*col*$^-$) strain (Fredericq, 1957, 1959). Such transfer takes place in the absence of other chromosomally determined characters, indicating that *C*-factors exist as extra-chromosomal elements.

In one case, that of the *C*-factor determining the production of colicin E1 (*colE*1$^+$), a chromosomal site has been proposed (Alföldi, Jacob, Wollman & Maze, 1958), with the conclusion that *colE*1$^+$ should be considered as an *episomal element*, in being able to take up either a chromosomal or a cytoplasmic location (Jacob, Schaeffer & Wollman, 1960). Alföldi *et al.* (1958) crossed several Hfr strains differing in *origine* and direction of transfer to the same F$^-$ strain, one parent only being colicinogenic for *E*1 (*colE*1$^+$) in each case. When the *C*-factor was in the Hfr donor, the frequency of its transfer varied with the Hfr strain used, and when the *C*-factor was in the recipient F$^-$ the latter was killed to an extent directly correlated with the ability of the Hfr strain to transmit *colE*1$^+$ when itself colicinogenic.

MATERIALS AND METHODS

Bacterial strains were all derivatives of *E. coli* K-12. Two recipient (F$^-$) strains were used; W677 (requiring threonine (*thr*$^-$), leucine (*leu*$^-$) and thiamine (*thi*$^-$)), and 748 (requiring proline (*pro*$^-$) and methionine (*met*$^-$), a recombinant of Hfr Cavalli and W1F$^-$), both strains being resistant to streptomycin (*str-r*) and to coliphage T6 (T6-r). The Hfr strains used were *Hayes* (H), *Cavalli* (C), *Reeves* 1 (R1), and P4X (equivalent to Hfr2 of Alföldi *et al.* (1958) and called J2 in this paper), all requiring methionine (*met*$^-$); also AB313 (Taylor & Adelberg, 1960) and P10 (equivalent to Hfr4 of Alföldi *et al.* (1959), this being called J4 in this paper), both strains being *thr*$^-$.*leu*$^-$.*thi*$^-$. All Hfr strains were sensitive to T6 (T6-s) and, with the exception of AB313, were streptomycin sensitive (*str-s*). They were all made resistant to M/500 sodium azide (*azi-r*). The '*origines*' and directions of transfer of these Hfr strains are shown in Fig. 1.

*Colicin factor E*1 (*colE*1$^+$) was transferred to all strains by overnight mixed culture at 37° with *E. coli* K-30. The K-12 strains were re-isolated by plating either on sodium azide (Hfr) or streptomycin (F$^-$). All non-colicinogenic strains were made resistant to the direct action of free colicin E1.

Hfr(*C*$^+$) × F$^-$(*C*$^-$) crosses were performed by mixing at 37° a logarithmic Hfr culture with a diluted, overnight culture of the F$^-$ strain, both at about 2 × 10^8 cells per ml., to give a ratio Hfr : F$^-$ of 1 : 20. Interrupted matings were performed by adding aliquots at

varying times up to 120 min. after mixing, to a suspension of T6 (10^{10} particles/ml.), shaking vigorously for half a minute, and after a further 10 min. incubation at 37°, diluting and plating on streptomycin nutrient agar. The F⁻ clones (*str*-r, T6-r) which appear after overnight incubation at 37° were then tested for the acquisition of colicin E1 factor, by overlaying with soft agar containing a colicin-sensitive indicator strain, and incubating overnight at 37°, when C^+ colonies produce zones of inhibition in the confluent lawn of indicator.

Hfr(C^-) × F⁻(C^+) and Hfr(C^-) × F⁻(C^-) crosses were performed by mixing at 37° logarithmic cultures of the Hfr strains with either logarithmic or diluted overnight cultures of the F⁻ strain, both again at *c.* 2×10^8/ml., to give a ratio of Hfr : F⁻ which varied from 2·5 : 1 to 100 : 1. At times up to 120 min. after mixing, samples were diluted and plated on streptomycin nutrient agar, to give a measure of the surviving F⁻ population.
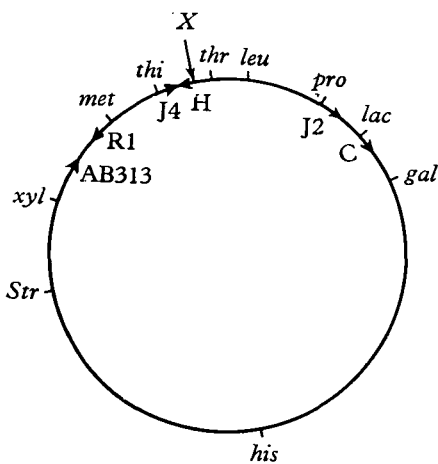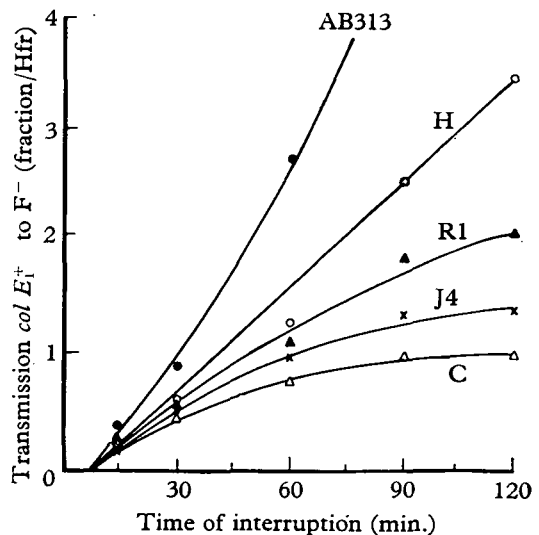

FIG. 1

FIG. 2

Fig. 1. Circular chromosome map of *Escherichia coli* K-12. The symbols on the outside of the circle represent bacterial markers, the symbol X denoting the suggested location of the *colE1* factor by Alföldi *et al.* (1958). The symbols inside the circle denote Hfr strains whose *origines* and directions of transfer are shown by the arrows on the circle.

Fig. 2. Kinetics of transfer of *colE1*⁺ from Hfr(*colE1*⁺) strains to W677F⁻. The individual curves represent the appearance of *colE1*⁺ in W677F⁻ when mated with various Hfrs at a Hfr : F⁻ ratio of 1 : 20.

RESULTS

Hfr(C^+)F × ⁻(C^-) crosses using W677 as F⁻ strain are shown in Fig. 2. The extent of transfer of C^+ to the F⁻ strain can be seen to vary with the Hfr strain used, and in most cases the proportion of F⁻ clones acquiring C^+ was in excess of the Hfr input, which was, however, one-twentieth the level of the total F⁻ population. Extrapolations of these curves all cut the time axis between 7 and 12 min. Crosses using 748 as a recipient strain were not essentially different to those of W677.

Hfr(C^-) × F⁻(C^-) crosses were carried out using Hfr Hayes × 748, in which the input of the Hfr strain varied from 2·5 to twenty-fold that of the F⁻ strain. The recipient 748 was

used either as a dilution of an overnight culture, or as a logarithmic culture. The results using overnight cultures are shown in Fig. 3a. It can be seen that a progressive increase in the ratio of Hfr : F⁻ produces a progressive lethal effect on the F⁻ cells. At multiplicities of 10 and less, the F⁻ population increases initially but falls after 30–60 min. At multiplicities greater than 10, lethality is seen before 15 min., and kills up to 99·5% of the initial F⁻ population. Logarithmic cultures of 748 showed similar, but less marked, effects, so that larger ratios of Hfr : F⁻ were required to produce the same lethal effect, a multiplicity of 100 : 1 being necessary to produce a lethal effect similar to that produced by a 15 : 1 ratio with overnight cultures, and multiplicities of around 20 : 1 producing effects obtained by a 5 : 1 multiplicity using overnight recipient cells. Fig. 3b shows the results of
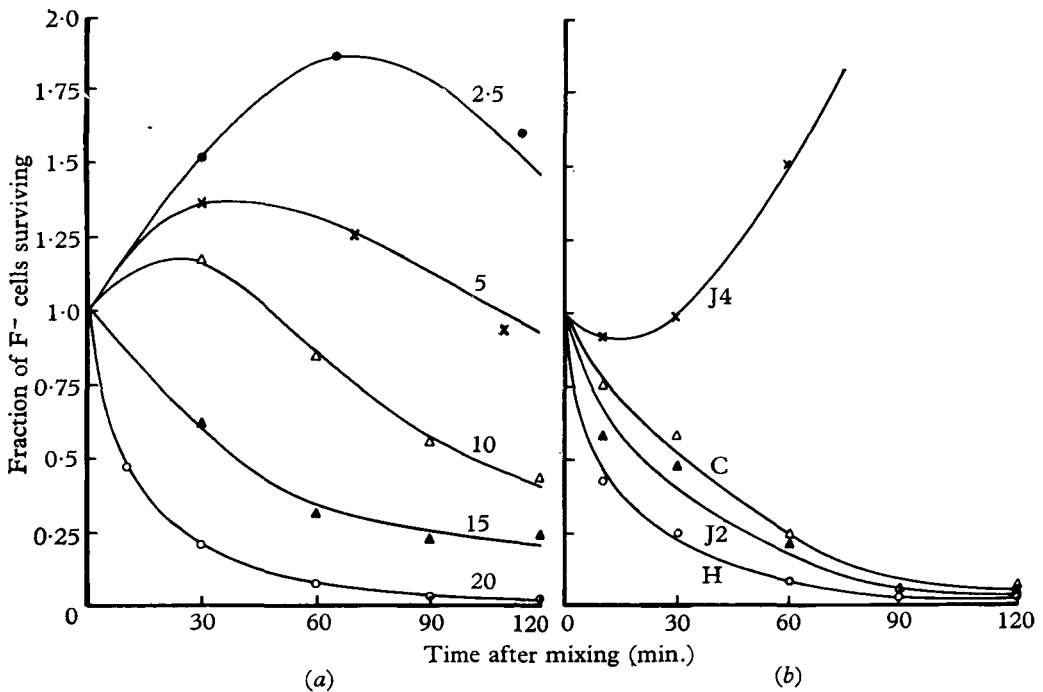

FIG. 3(a)   FIG. 3(b)

Fig. 3a. Survival curves of 748F⁻ when mated with HfrH at ratios of Hfr : F⁻ from 2·5 to 20, as shown on the individual curves.

Fig. 3b. Survival curves of 748F⁻ when mated at a Hfr : F⁻ ratio of 20 : 1 with the various Hfr's designated on the individual curves.

crossing various Hfr strains with overnight cultures of 748F⁻ as recipients, at a standard multiplicity Hfr : F⁻ of 20 : 1. A marked difference was seen with the Hfr parent chosen. Strains H, C and P4X (= J2) had pronounced lethal effects, which were most marked with H. Strain P10 (= J4) produced no overall lethal effect, although there was initially a slight drop in F⁻ count, which finally increased to almost twice the initial value.

Crosses using W677 as recipient gave similar but less well-marked effects. Overnight W677 cultures when mixed with HfrH to give an Hfr : F⁻ ratio of 50 : 1 produced curves resembling those of Fig. 3a for 748 at multiplicities of 5 to 15. Variations were found in the response of W677 in successive experiments, the curves sometimes showing a continuous decrease in F⁻ counts (as $M = 15$, Fig. 3a) but at other times producing initial increases

(as $M = 5$, Fig. 3a). However, final count at 120 min. was always below the initial level, and varied from about 0·2 to 0·8. Similar effects were found using HfrC, R1 and P4X (final values 0·24 to 0·76). In contrast, using HfrJ4 ($= P10$), the F$^-$ count increased steadily at all times and at 120 min. was between 2·4 and 4·4 times the initial value.

Lethal effects using logarithmic W677 were even less well-marked. With HfrH, C and J2, the F$^-$ count increased for the first 60 min. and then fell to values of from about 0·8 to 1·5. An overall decrease at 120 min. was frequently, but not always, obtained. In contrast, in crosses using HfrJ4, the F$^-$ count increased continuously and at 120 min. was invariably between 3·5 and 5 times the initial level. Hfr(C^-) $\times$ F$^-$(C$^+$) crosses were carried out using W677 *colE1*$^+$ only, as the F$^-$ strain. Essentially similar results were obtained to those using non-colicinogenic recipients as quoted above. There was no difference in the extent of lethal effects.

DISCUSSION

The extent of transfer of *colE1*$^+$ from Hfr to F$^-$ strains varied with the Hfr strain used (Fig. 2), as found by Alföldi *et al.* (1958). However, it can be seen from a comparison of Figs. 1 and 2 that there is no correlation between the level of transfer of C^+ and the 'origine' or transfer direction of these particular Hfr strains. which would support the concept of a chromosomal location for *colE1*$^+$ at the position on Fig. 1 shown as 'X', as suggested by Alföldi *et al.* (1958). This is confirmed by the observation that the 'time of entry' of the C factor is between 7 and 12 min. for *all* Hfr strains. No *single* chromosomal location is situated 7–12 min. from the 'origines' of all five Hfr's. It is concluded that *colE1*$^+$ passes from Hfr to F$^-$ as a non-chromosomal element independently of chromosomal transfer, which occurred to a normal level during the course of these experiments. Transfer to fractions of the F$^-$ population in excess of the input Hfr must be explained either by the known multinucleate nature of *E. coli* cells (see Hayes, 1960), permitting one Hfr cell to transfer C^+ to several independent F$^-$ cells, or the ability of the C^+F$^-$ cells to multiply, during the course of these experiments. Separate experiments confirmed that C^+ is not transferred from F$^-$ to F$^-$ cells, but that an unmated F$^-$ population increases fivefold during the course of the experiment, after an initial 20 min. lag.

The lethal effects cannot be due to a putative C locus, transferred from a C^- to a C^+ strain, as postulated by Alföldi *et al.* (1957, 1958), since similar lethal effects occur with strain 748 in the absence of any C factor (Figs. 3a and b). These lethal effects occur at a high ratio of Hfr to F$^-$ cells (such as was used in the experiments of Alföldi *et al.*) and appear to result from multiple conjugation or transfer. It should be noted that strain J4 is without lethal effects, as found by Alföldi *et al.* (1958), and strains H and J2 produce lethality to the same marked degree (Fig. 3b).

The standard recipient (W677) was not as amenable to this lethal zygosis as 748. However, lethal effects were produced, and although not always quantitatively reproducible, invariably showed decreases in F$^-$ survivors in crosses with strains like HfrH and HfrJ2, but never with strain J4, making it probable that this effect is analogous to, but less pronounced than, that obtained with 748. A variety of other F$^-$ recipients (C600, P678, W1, W945) gave similar results to W677.

It is concluded that *colE1*, and probably other C factors, are best regarded as plasmids (Lederberg, 1952) rather than episomes, until a more direct association of these elements with the chromosome is demonstrated. A chromosomal location could, however, be inferred from the ability of some of these C factors to mediate the transfer of chromosomal characters (Clowes, 1961; Ozeki & Howarth, 1961).

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