SHORT PAPER

A model relating the replication and expression of colicin factor E2-P9

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SUMMARY

The observed dependence of lacuna counts on the growth phase of colicinogenic cultures suggests a relationship between the probability of colicin synthesis, and the number of colicin factors, per cell.

Colicin synthesis by individual bacteria is measurable by lacuna counts (Ozeki, Stocker & de Margerie, 1959). In this technique, the culture is usually chloroformed and then plated in overlays with a colicin-sensitive indicator strain. After incubation, small clearings ('lacunae') appear in the indicator lawn which are presumed to be produced by cells synthesizing colicin at the time the culture was chloroformed. We find that the percentage of lacuna-forming cells (% LFC) in different cultures of a strain carrying colicin factor E2-P9 (ColE2-P9) is highly variable and depends on the growth phase of the cells. This variability may arise directly from the manner in which the expression and replication of the Col factor is regulated, as will be seen from the model suggested below.

The present experiments concerned *Escherichia coli* K12 (strain KH293, i.e. AB1157 E2-r) carrying ColE2-P9 which forms unequivocal lacunae. The % LFC in cultures of this strain changed dramatically at different stages of growth. Its minimum value was about 0·01 % and was found in exponentially growing cultures with less than 5×10^7 cells/ml. It increased rapidly after the colony count reached 5×10^7/ml (Fig. 1). After overnight incubation, the value could be as large as 10 %. The most significant finding for the proposed model was that when such stationary cells were allowed to grow in fresh medium, the % LFC halved in successive generations (Fig. 2) until either it reached the minimum of 0·01 % or the colony count exceeded 5×10^7/ml when it again began to increase (Fig. 1). The model suggested here relates all these changes in % LFC to the number of copies of ColE2-P9 per cell and to the way in which the structural gene for the colicin becomes expressed.†

We note first that the number of copies of ColE2-P9 per chromosome varies with the stage of growth of the culture. Thus, dye-buoyant density gradient centrifugation using ethidium bromide showed that the % Col factor DNA typically increased from 3 % at a

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† Colicin synthesis is also affected by nutritional factors. Lacunae produced by cells from overnight cultures may be relatively dim but after incubation in fresh medium, the lacunae become clear and the % LFC may show a rise preceding the decline shown in Fig. 2. These changes are presumably produced non-specifically by starvation of the cells in overnight culture.
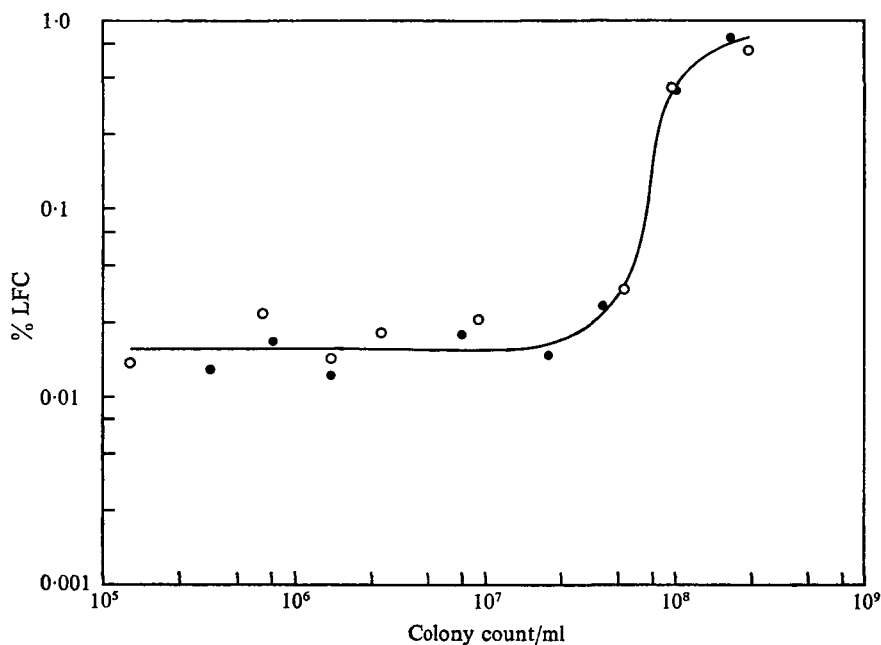
Fig. 1. Increase in % LFC (ordinate) with increase in colony count (abscissa). An 18 h unshaken culture of strain KH293 was diluted 10^{-6} in nutrient broth. After 4·5 h incubation at 37 °C, samples were removed at 30 min intervals for lacuna and colony counts. The figure shows the results for two independent experiments.

colony count of 10^8/ml to 10 % after a further 120 min incubation when the count was 8×10^8/ml. Since the molecular weight of ColE2-P9 is 5×10^6 (Bazaral & Helinski, 1968), these percentages correspond, respectively, to 14 and 45 copies per chromosome. That is, the replication of ColE2-P9, like that of ColE1-K30 (Bazaral & Helinski, 1970), is under relaxed control. For the purpose of the model, the relevant point is that the number of copies per chromosome increases on entering the stationary phase and decreases when stationary phase cells begin to grow in fresh medium.

These observations can be related to % LFC by postulating

$$\% \, \mathrm{LFC} = Cp,$$

where C is the number of copies of ColE2-P9 and p is the constant probability per copy of causing its host cell to form a lacuna. It is suggested that:

(a) The % LFC increases in the stationary phase because C increases. Although such an increase can readily be demonstrated, as described above, the estimates of the % Col factor DNA are not sufficiently precise to show unequivocally that the % LFC is proportional to C.

(b) For the average stationary phase cell, C may be as much as 1000 times ($= 10\%/0·01\%$) greater than for the average exponential phase cell from cultures where the % LFC is a minimum. This is not unreasonable, judging from the number of copies found here and with ColE1-K30 (Clewell, 1972).

(c) When such stationary phase cells are grown in fresh medium, the % LFC halves in successive bacterial generations because C is also halving. That is, none of the copies in each cell replicates until either C has returned to the value corresponding to the minimum of 0·01 % LFC found in exponential cultures or C begins to increase after the colony count reaches 5×10^7/ml.
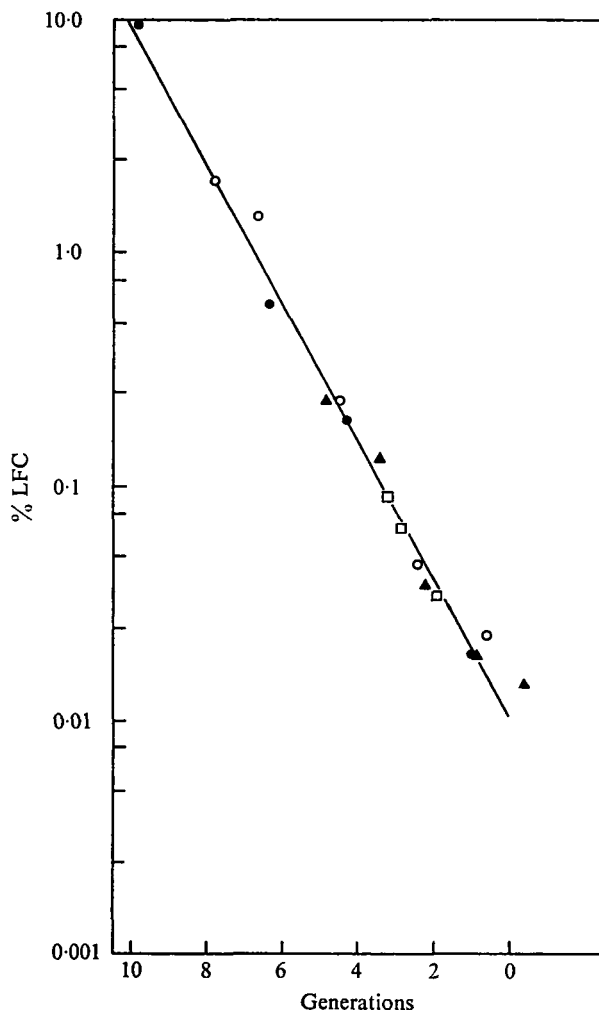
Fig. 2. Decrease in % LFC (ordinate) on diluting stationary phase cells in fresh medium. Data from four independent experiments normalized by the number of generations (abscissa) required to reach the minimum of 0·01 % LFC. An overnight unshaken culture of strain KH293 was diluted 10^{-3} in nutrient broth. Samples were removed at intervals for lacuna and colony counts. The colony count was kept at less than 5×10^6/ml by dilution at intervals in fresh medium.

(*d*) Each copy of ColE2-P9 is independent of all other copies in its host cell with respect to lacuna formation (presumably, with respect to expression of the structural gene specifying the colicin). This follows from the constancy of *p*. If this interpretation is correct, the event determining whether or not the structural gene is expressed occurs at the level of that gene, or its Col factor, as distinct from a generalized change in the host cell causing expression of all the copies it carries.

The model therefore brings together several aspects of the behaviour of this colicin factor. Further tests are needed to exclude alternative explanations of the data: e.g. that Fig. 2 reflects the behaviour of cells pre-destined to lacuna formation in stationary phase cultures and which never divide in fresh medium. The model also bears on the behaviour

of ColE2-P9 in the presence of chloramphenicol. This increases the % LFC in both u.v.-irradiated (Ben-Gurion, 1965) and unirradiated cultures carrying ColE2-P9 (Ben-Gurion, 1970; Kennedy, 1971). Such observations can be related to the number of copies of the Col factor by the predictions of the model.

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