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Studies on the Colicinogenic Factor in *Escherichia coli* K 12 Induction of Colicin Production by Mitomycin C

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SUMMARY

Mitomycin C induces the production of colicin in *E. coli* K 30 and colicinogenic strains of K 12. The production of colicin seems to involve *de novo* synthesis of protein in a fraction of the population of a colicinogenic strain. The proportion of this fraction is increased by treatment with inducing agents. The optimal conditions were demonstrated by two methods, lacunae counting and assay of the supernatant. By both methods the optimal conditions were found to be the same.

INTRODUCTION

Mitomycin C, an antibiotic isolated from *Streptomyces caespitosus*, selectively inhibits the synthesis of deoxyribonucleic acid (DNA) in *Escherichia coli* (Shiba *et al.*, 1959). Additional studies have revealed that DNA synthesis which has been inhibited by mitomycin C treatment can be promptly restored by infection of the cells with bacteriophage T2 (Sekiguchi and Takagi, 1959, 1960a). Most of the bacteriophages which multiply in the presence of mitomycin C are non-infectious (Sekiguchi and Takagi, 1960b), but these phages have the ability of multiplicity reactivation, cross reactivation and mutual exclusion (Okubo, 1962). Mitomycin C also affects genetic mechanisms in *E. coli* exerting a mutagenic action, increasing the recombination rate (Szybalski, 1958; Iijima and Hagiwara, 1960; Yuki, 1962), and inducing phage development in a lysogenic strain of *E. coli* K 12 (Otsuji *et al.*, 1959).

This communication presents evidence that mitomycin C can induce colicin production in colicinogenic strains of *E. coli*. Preliminary results have been reported elsewhere. (Iijima, 1961).

MATERIALS AND METHODS

Organisms: The strains used in these experiments are described in Table 1. *E. coli* K 12 and its mutants were originally isolated and supplied by Dr. J. Lederberg and have been propagated in our laboratory. *E. coli* K 30 and TR 16 were supplied by Dr. P. Fredericq. Colicinogenic strains of *E. coli* K 12 were isolated in this la-

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boratory by mixing the strain with appropriate donor strain.

Table 1. Summary of Strains Used

strain	characteristics
K 30	prototroph, colicinogenic strain.
W 1177	Threonine, leucine, thiamin requiring, lactose, galactose, maltose, nonfermenting, streptomycin resistant strain derived from K 12.
TR 16	W 1177 colicinogenic strain.
Y 70	Threonine, leucine, thiamin requiring, lactose nonfermenting streptomycin sensitive strain.
TR 706	Y 70 colicinogenic strain.
WR 110	Colicin resistant strain derived from W 1177.

Media: The peptone broth used contained the following constituents in distilled water: Peptone (Kyokuto) 1 %, meat extract (Kyokuto) 1%, NaCl 0.2%. A synthetic medium used, had the following composition: Glucose 3 gm, $(\text{NH}_4)_2\text{HPO}_4$ 2.5 gm, KH_2PO_4 0.1 gm, NaCl 5 gm, Sodium glutamate 2 gm and distilled water 1 litre. The pH was adjusted to 7.2 with NaOH. Glucose was sterilized separately and mixed with the medium after cooling.

Methods: *E. coli* K 30 (or TR 706) which is colicinogenic and streptomycin sensitive strain was grown at 37°C. The cells were collected by centrifugation and resuspended in the synthetic medium. After treatment with mitomycin C (Kyowa Hakko Co.) or ultraviolet light (Toshiba GL-15), the colicin titer was assayed by two different methods, *i.e.*, supernate assay and lacunae counting (Ozeki *et al.*, 1959).

Supernate assay: After treatment, cells were incubated a further 1.5 hours at 37°C to allow release of colicin into the medium. As shown below, colicin production reached a maximum after 1.5 to 2 hours. The cells were centrifuged, and the supernatant was used for the assay. A colicin sensitive and streptomycin resistant indicator strain, W 1177, was mixed with the supernatant and after two hours, the number of survivors of the indicator strain were counted by spreading the mixture on nutrient agar plates containing streptomycin (100 $\mu\text{g}/\text{ml}$).

Lacunae counting: After treatment, the cells were diluted and mixed with a colicin sensitive and streptomycin resistant indicator, W 1177, together with 3 ml of 0.7% soft agar. The mixture was poured onto nutrient plates containing streptomycin (100 $\mu\text{g}/\text{ml}$). After 18 hours incubation, a number of small clear spots, "lacunae", appeared on the plate in the confluent growth of the indicator strain. These lacunae were not observed when TR 16, which is immune to colicin or WR 110, which is resistant to it were used as indicators.

RESULTS

The effect of colicin in the supernatant of K 30 cultures on two strains, W 1177 and TR 16, is shown in Fig 1. TR 16 differs from W 1177 only in its colicinogenic property.

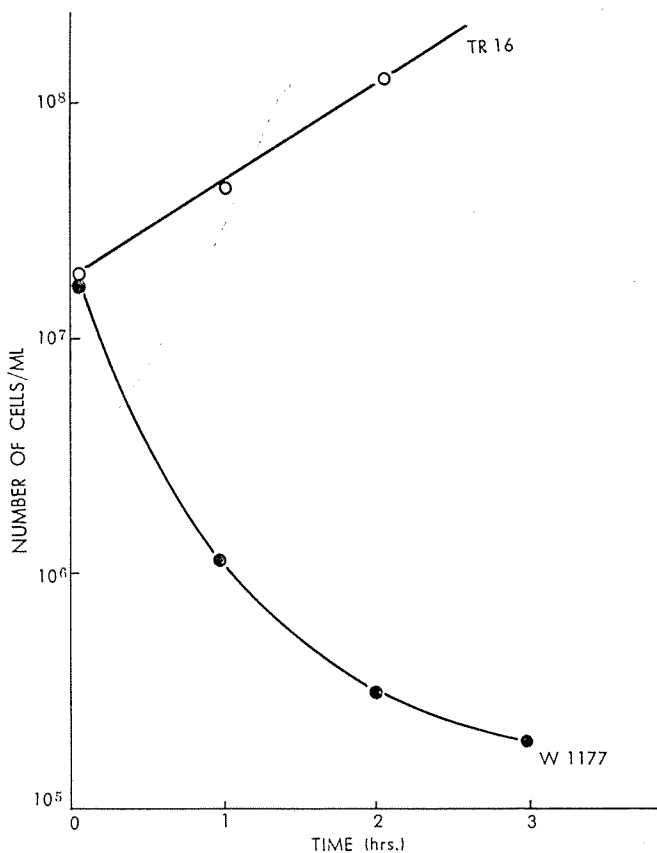


Fig. 1. Killing Action of Colicin on Two Strains

Escherichia coli K30 was treated with mitomycin C ($0.1 \mu\text{g/ml}$) for 10 min. The treated culture was incubated for 2 hours at 37°C . After incubation, the supernatant of the culture was collected by centrifugation. W 1177 or TR 16 was mixed with the supernatant and the number of survivors was counted.

Colicin production varied with the dose of mitomycin C or ultraviolet light used. Optimal conditions for colicin production were obtained by treating cells in the synthetic medium with $0.1 \mu\text{g/ml}$ of mitomycin C for 15 min. or by 30 seconds irradiation from an ultraviolet lamp at a distance of 45 cm. The optimal condi-

tions as judged from supernatant assay were the same as those judged from lacunae counting (Fig.2, Fig.3). The rate of synthesis of colicin after induction can be

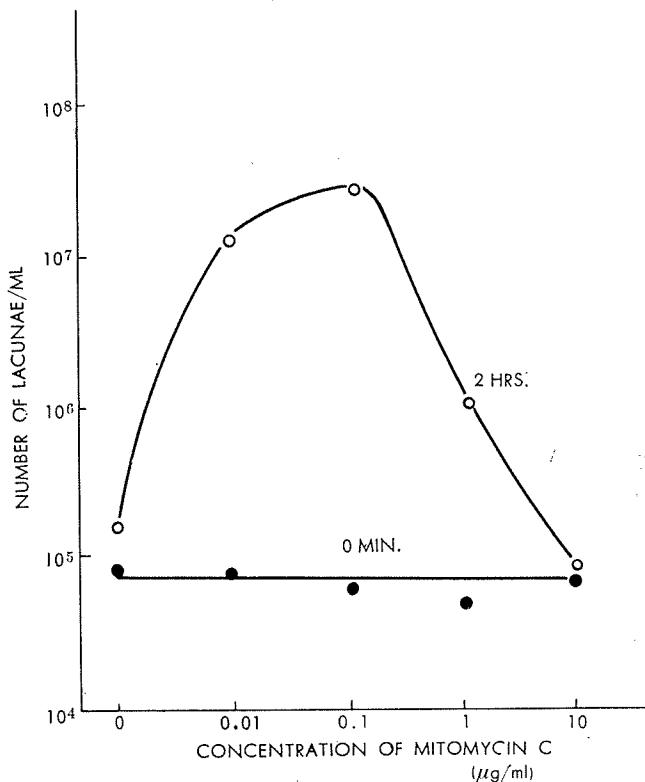


Fig. 2. The Effect of the Mitomycin C Concentration on Lacunae Formation

A colicinogenic strain was treated with various concentrations of mitomycin C. Lacunae counting was carried out immediately after treatment and after 2 hours incubation at 37°C.

followed by the lacunae counting method. Figure 4 shows the increase in lacunae after treatment with inducing agents. An untreated culture produces lacunae spontaneously at a rate of 10^{-3} per cell in a population. Immediately after treatment, the culture produced the same number of lacunae as the untreated culture. However, when the treated culture was incubated at 37°C in broth the lacunae count increased rapidly and reached a maximum after 2 hours. In the control culture the lacunae count did not increase during incubation.

These results show that colicin synthesis occurs spontaneously at definite rate (about 8×10^{-4} per cell in K 30 population), in a population of colicinogenic

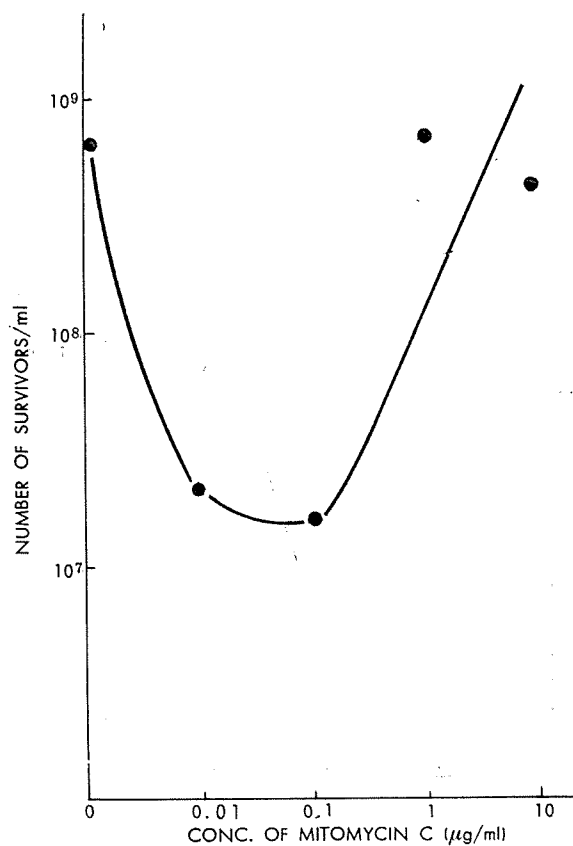


Fig. 3. The Effect of the Concentration of Mitomycin C on the Induction of Colicin Production

A Colicinogenic strain was treated with various concentrations of mitomycin C. After 2 hours incubation, each of the supernatants was mixed with an indicator strain, W1177 and the number of survivors was counted 2 hours later.

strain, but after the treatment with inducing agents, other fractions in the culture begin to synthesize colicin. When chloramphenicol was added at various periods after induction, there was a decrease in the rate of increase in lacunae, depended on when the chloramphenicol was added (Fig.5). These results suggest that the synthesis of colicin involves *de novo* synthesis in the induced cells.

DISCUSSION

The induction of colicin production in a colicinogenic strain of *E. coli* was first observed in *E. coli* ML and later in other strains after treatment with ultra-violet light (Jacob *et al.*, 1951; Fredericq, 1954; Hamon and Lewe, 1955).

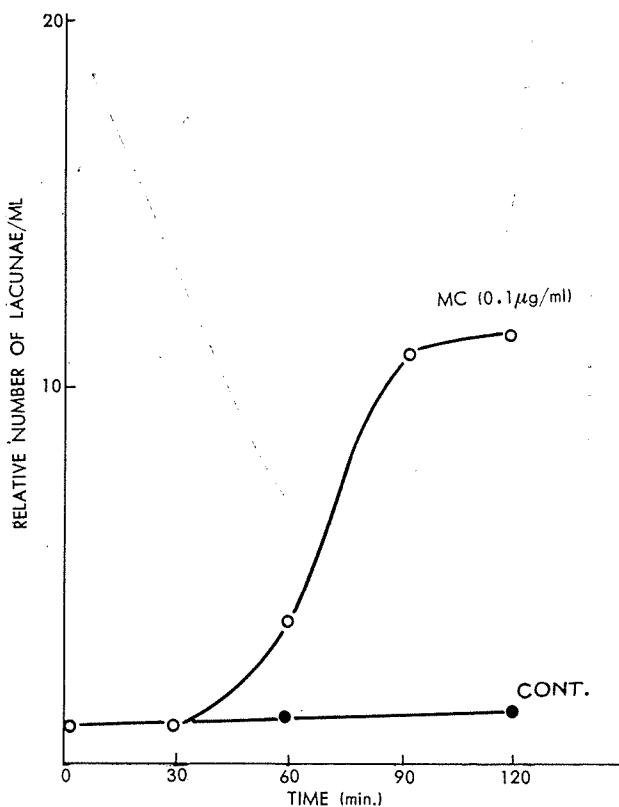


Fig. 4. Lacunae Formation after Treatment with Mitomycin C

Mitomycin C is of biological interest, because of its antineoplastic activity, selective inhibition of DNA synthesis, ability to induce the development of prophages in lysogenic bacteria, mutagenic action and ability to induce the production of colicin in colicinogenic bacteria. These characteristic effects are similar to those of ultraviolet light. Furthermore, UV and mitomycin C have similar effects on the chromosomes of *E. coli K 12* and affect the recombination process in the recipient cells (Yuki, 1962). When *Hfr* donors are exposed to UV or mitomycin C, and mated with untreated *F-*, the treated *Hfr* donor decrease in fertility exponentially as a function of the dose. The decrease in the rate of recombinant formation is faster than the decrease in the rate of transfer of the colicinogenic factor. As was shown previously, transfer of the colicinogenic factor is not so much affected by this treatment (Iijima, 1961). Lavallo and Jacob (1961) suggested that the colicinogenic factor was resistant to disintegrations of P^{32} incorporated into the cell, and assumed that the colicinogenic factor contained less nucleic acid. The

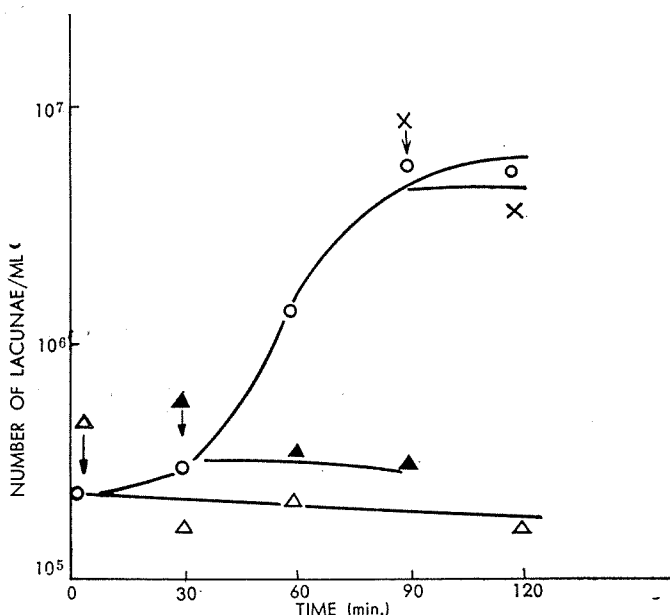


Fig. 5. The Effect of Chloramphenicol on Lacunae Formation
Chloramphenicol was added at the time indicated by the arrows.

difference in the sensitivity of the chromosomal character and the colicinogenic factor may reflect a difference in the structure of the colicinogenic factor.

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