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Specific Inhibition of Cell Division by Colicin E_2 Without Degradation of Deoxyribonucleic Acid in a New Colicin Sensitivity Mutant of *Escherichia coli*

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A new class of colicin sensitivity mutants of *Escherichia coli* was isolated whose cell division was specifically inhibited by colicin E_2 without detectable degradation of deoxyribonucleic acid (DNA) at 30 C. The mutant could not form colonies in the presence of colicin E_2 but recovered colony-forming ability by trypsin treatment even after prolonged incubation with the colicin. Addition of colicin E_2 to the exponentially growing mutant inhibited cell division completely but did not induce degradation of DNA into cold acid-soluble materials nor any breakage of DNA strands. Synthesis of DNA in the mutant was not inhibited, and long filamentous cells with multiple nuclear bodies were formed by the action of colicin E_2. Degradation of ribosomal ribonucleic acid and development of prophage λ, both of which were induced by colicin E_2 in the sensitive cells, did not occur in the mutant. At the elevated temperature, however, the mutant was found to undergo colicin-induced degradation of DNA. No differences in ultraviolet light nor drug sensitivities were observed in the mutant compared to the parent *E. coli*. The data suggested that colicin E_2 had a specific inhibitory effect on cell division of *E. coli* that was not a consequence of DNA degradation.

Colicin E_2 is a simple protein of molecular weight 62,000 which is produced by *Enterobacteriaceae* harboring episomic colicin E_2 factor (8). The main feature of its biochemical action on the sensitive cells of *Escherichia coli* is the induction of rapid and extensive degradation of deoxyribonucleic acid (DNA) (3, 10, 13, 16), but only limited degradation of ribosomal ribonucleic acid (RNA) (12) and λ-prophage induction (7). Investigation in a subcellular system showed that colicin E_2 had a dissociating activity on the isolated DNA-membrane complex of *E. coli* (2). On the other hand, appropriate amounts of colicin E_2 have been known to inhibit cell division of sensitive cells without marked inhibition of protein synthesis and to result in the appearance of long filamentous cells as is found with other DNA-attacking agents. Although the inhibition of cell division seemed to be a direct consequence of the DNA degradation by colicin E_2, these two main effects of colicin E_2 may not be necessarily interlinked in such a sequence. A purpose of the experiments described in this paper was to determine whether DNA degradation is required for killing (i.e., inhibition of cell division) by colicin E_2.

To solve this problem, we tried to isolate a new class of mutant that lost its colony-forming ability without suffering DNA degradation by colicin E_2. The desired mutant was isolated, and investigations on the action of colicin E_2 on this mutant revealed that colicin E_2 inhibited cell division specifically without any detectable DNA degradation.

MATERIALS AND METHODS

Organisms. A thymine-requiring auxotroph of *E. coli* K-12 strain W2252 (Hfr, *met*$^-$, λ^s) was used as the parent, sensitive strain. A colicin-tolerant mutant, no. 617 (*tolA*), derived from *E. coli* strain W607 (A. Bernstein et al., Bacteriol. Proc., p. 50, 1971), and recombination-deficient mutants, strains AB2470 (*recB21*) and JC5474(*recC22*) (18), were

tested for trypsin reversibility of colicin E_2-induced death. BF23 phage was used to test the receptor for E-group colicins. Phage λ was obtained by induction of *E. coli* K-12(λ) by ultraviolet light (UV); λ_{cI-c47} and $\lambda_{vir\ (v1, v2, v3)}$ were used to check lysogeny of the organisms.

Media. Cells were cultured aerobically at 30 C in 2% nutrient broth (Kyokuto, Tokyo, Japan) containing 50 μg of thymine per ml. Broth-agar medium contained 1.5% agar in the above medium. Broth-agar medium containing trypsin (trypsin plate) to score the cells reversibly killed by colicin E_2 was prepared by smearing 0.1 ml of a solution of crystalline trypsin (0.8 mg/ml) on the 20-ml solidified broth-agar plate. Broth-agar medium containing colicins E_2, E_3, or K, or phage BF23 was prepared similarly by spreading 0.1 ml of the colicin solution (10 μg/ml) or suspension of the phage (5×10^9 particles/ml).

Procedure for isolation of the mutant. The parent bacteria growing exponentially at 37 C were suspended in 0.05 M potassium phosphate buffer (*p*H 6.0) at a cell concentration of 5×10^8 cells/ml and treated with 30 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml for 60 min at 37 C. The suspension was diluted with fresh broth and grown for 3 to 5 hr at 30 C. Purified colicin E_2 was added at a final concentration of 0.2 μg/ml, and the culture was incubated for 1 hr at 30 C. The cells treated with colicin E_2 were spread on the trypsin plates which were then incubated at 37 C overnight. Colonies that appeared on this plate were transferred to the broth-agar plates containing colicin E_2 by replica plating to check the sensitivity to colicin E_2. The strains that showed no growth on the colicin-containing plates were selected, and the trypsin reversibility of these strains from death by colicin E_2 was further confirmed. Ten strains of 132 selected colonies tested showed the property of "trypsin-reversible death" by colicin E_2.

Isolation of λ-lysogens and prophage induction by colicin E_2. To obtain lysogenic strains of W2252*thy*$^-$ and the mutant DR312, survivors from phage λ infection at a relatively high multiplicity were isolated and tested for their adsorption capacities of phage λ by λ_{vir} and their immunity to λ_{cI}. Strains resistant to λ_{cI} but sensitive to λ_{vir} were selected as lysogens. All of the isolated lysogens derived from strain DR312 were found to be induced by UV but not by colicin E_2.

To measure efficiency of prophage induction by colicin E_2, lysogenic strains growing in the nutrient broth at 30 C were treated with various amounts of the colicin at 30 C for 40 min. The cells were then plated on the nutrient broth-agar plates with *E. coli* strain W2252*thy*$^-$ as an indicator, and plaques were counted after overnight incubation at 37 C.

UV sensitivity test. Growing cells of strains W2252*thy*$^-$ and DR312 were centrifuged and washed with 0.8% NaCl. The washed cells were resuspended in 5 ml of 0.8% NaCl at cell densities of 5×10^8 cells/ml and irradiated in petri dishes (8.4 cm in diameter) with a UV lamp (Toshiba GL-10) at a distance of 35 cm.

Microscopy observations. The total number of cells was counted by using a Petroff-Hauser counting chamber under a phase-contrast microscope. To observe the distribution of nuclear bodies in the filamentous cells of strain DR312 formed by colicin E_2, nuclear staining was performed by hydrolyzing fixed cells with 1 N HCl at 60 C for 8 min, followed by staining with Giemsa reagent.

Measurements of degradation and synthesis of DNA and other macromolecules in the cells. Measurement of the DNA degradation induced by colicin E_2 was described previously (3). Degradation of ribosomal RNA was measured similarly with the cells grown in medium containing uracil-2-^{14}C (10 μg/ml, 3.3 mCi/mmole) and chased with medium containing nonradioactive uracil. DNA synthesis was followed by the incorporation of thymine-2-^{14}C (50 μg/ml, 0.83 mCi/mmole) into cold 5% trichloroacetic acid-insoluble materials. RNA synthesis and protein synthesis were followed by the incorporation of ^{14}C-uracil and of methionine-1-^{14}C (20 μg/ml, 5.3 mCi/mmole), respectively.

Alkaline sucrose density gradient sedimentation for detecting breaks of DNA by colicin E_2 was accomplished as follows. Cells were lysed by incubation with 10 mM ethylenediaminetetraacetate (EDTA) and 0.1 mg of lysozyme per ml at 0 C, and then 0.5 volume of 1.0 M NaOH was added to the lysate. Two-tenths milliliter of this sample was placed on a 5-ml gradient of 5 to 20% (w/v) sucrose containing 0.3 M NaOH, 1 mM EDTA, and 1.0 M NaCl and centrifuged at 30,000 rev/min for 110 min with the RPS40 rotor of a Hitachi ultracentrifuge. Fractions were collected from the bottom of the tube on 2-cm filter disks and washed with cold 5% trichloroacetic acid and ethanol before being counted in a scintillation counter.

Preparation of colicin E_2 and other chemicals. Purified colicin E_2 and colicin E_3 were prepared mainly by the method of Herschman and Helinski (8). Purified colicin K was a gift from M. Matsuhashi, Institute of Applied Microbiology, Tokyo. "Multiplicity" of the added colicins to the cells was calculated as described by Nomura (10).

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine was purchased from Tokyo Kasei Co., Tokyo, Japan. Thymine-2-^{14}C, uracil-2-^{14}C, and DL-methionine-1-^{14}C were from Daiichi Pure Chemicals Co., Tokyo, Japan. Crystalline trypsin was from Mochida Pharmaceutical Co., Tokyo, Japan.

RESULTS

Isolation of a mutant able to be rescued by trypsin from killing by colicin E_2. It had been known that trypsin digestion of colicin K-treated cells resulted in rescue of those cells from death even after prolonged incubation with the colicin (9). A similar trypsin-reversible state of killing was also observed with colicin E_2, but this state continued for only very short period, and the colicin E_2-treated cells were converted rapidly to the state of trypsin-

irreversible death because of irrepairable DNA damage due to extensive degradation (1, 15). If mutants exist which lose their colony-forming ability by colicin E_2 without DNA degradation, they may be expected to remain in the state of trypsin-reversible death even after a long exposure to colicin E_2. This possibility was adopted as a screening condition to obtain the mutant, and clones that could form colonies on trypsin-containing plates after incubation with colicin E_2 (condition A) but could not form colonies on colicin E_2-containing plates (condition B) were selected. Parent sensitive cells could not recover their viability under condition A, and colicin E_2-resistant or -tolerant mutants could form colonies under condition B.

E. coli strain W2252*thy*$^-$ was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and grown for segregation of mutants. Colicin E_2 was added to the culture at a high dose, and further incubation was performed for 1 hr at 30 C. A portion of the incubated cell suspension was spread on nutrient broth-agar plate containing trypsin and incubated overnight at 37 C. Colonies that appeared on this plate might contain colicin E_2-resistant and -tolerant mutants and the mutants that had been rescued from the colicin E_2 killing by trypsin in the plate, whereas almost all cells (more than 99.9%) having colicin sensitivity similar to that of the parent were irreversibly killed owing to DNA degradation during the incubation with colicin E_2. These colonies were then selected under condition B by replica plating on nutrient broth-agar plates containing colicin E_2. Strains that showed resistant growth in the presence of colicin E_2 were discarded as resistant or tolerant mutants and those that proved to be sensitive under this condition were stocked on nutrient broth-agar slants. Each of the strains thus selected was examined for viability under condition A and condition B, and two representatives, strains DR312 and TR22, with the property of trypsin-reversible death by colicin E_2, were selected for further study.

As shown in Table 1, almost 100% of the colicin E_2-treated DR312 cells formed colonies on the plates containing trypsin, but the cells treated with colicin E_3 or K could not be rescued by trypsin at all. Strain DR312 formed no colonies in the presence of colicins E_2, E_3, or K, or phage BF23, suggesting that the strain was sensitive to those colicins under such conditions. The sensitive parent, strain W2252*thy*$^-$, was not rescued by trypsin from the colicin death, whereas a resistant mutant, strain R-3, and a tolerant mutant, strain R-7, obtained during the course of this study, and a *tolA* mutant, strain W607-617, showed remarkable growth on the plates containing colicin E_2. Thus strain DR312 might be assumed to be a completely reversible strain specific for colicin E_2 and not a resistant nor tolerant mutant.

On the other hand, strain TR22 showed partial reversibility to colicins E_2 and E_3. As shown in Table 1, about 65% of both the colicin E_2-treated and colicin E_3-treated TR22 cells formed colonies on the trypsin plates. Furthermore, strain TR22 was found to have a partial resistance to phage BF23. When TR22 cells were spread on the agar plate previously smeared with phage BF23 suspension, very minute colonies (about 70% of the initial cell number) appeared after 2 days of incubation at 37 C. Plaques of this phage could not be observed with strain TR22 as an indicator lawn at all. These results suggested that the "reversible" property of strain TR22 might be due to a defect in the receptor structure which was common for E group colicins and phage BF23. This consideration made us choose strain DR312 for the following experiments. In these experiments, no trypsin reversibility could be observed with colicin K. This might be due to the irreversible damage to cells by the very high dose of colicin K used.

Effect of colicin E_2 on growth of DR312. The effect of the addition of colicin E_2 on the viability of the exponentially growing strain DR312 was observed by spreading colicin-treated cells on trypsin plates and counting the number of colonies. Increase in viable cell number of strain DR312 stopped immediately after the addition of 0.1 μg of colicin E_2 per ml (multiplicity = about 120), and the number was retained constantly for long period (Fig. 1). Slow decrease in the viable cell number occurred 100 min after the colicin challenge, but at a lower dose of the colicin (multiplicity = 60) it remained constant without decrease for more than 3 hr. In contrast, the addition of the same amount of colicin E_2 to growing *E. coli* strain W2252*thy*$^-$ resulted in an instant decrease in viability (i.e., colony-forming ability on the trypsin plates). Increase in the culture turbidity of strain DR312 was not stopped by colicin E_2. Microscopy observation of the colicin E_2-treated culture of DR312 showed that increase in total cell number also stopped concomitantly, and almost all cells became filamentous after incubation with the colicin. These results indicated that colicin E_2 inhibited cell division of strain DR312 without trypsin-irreversible killing.

TABLE 1. *Trypsin rescue of Escherichia coli strains from killing by colicin E_2*

Strain[a]	Colicin or phage	Initial number of cells ($\times 10^6$ cells/ml)	Trypsin-rescued cells[b] ($\times 10^6$ cells/ml)	Trypsin reversibility (%)	Resistant cells ($\times 10^6$ cells/ml)	Sensitivity[c]
W2252*thy*$^-$	E_2	252	0	0	0	s
	E_3	132	0	0	0	s
	K	212	0	0	0	s
	BF23	800			0	s
DR312	E_2	238	239	100	0	s
	E_3	151	0	0	0	s
	K	208	0	0	0	s
	BF23	532			0	s
TR22	E_2	125	82	65.6	0	s
	E_3	250	163	65.2	0	s
	K	250	0	0	0	s
	BF23	523			350[d]	pr
R-3	E_2	313	349		302	r
	E_3	256	310		267	r
	BF23	431			405	r
R-7	E_2	175	245		182	r
	E_3	154	202		166	r
	BF23	221			0	s
W602-617 (*tolA*)	E_2	310	380		305	r
AB2470 (*recB21*)	E_2	127	2	0.2	0	s
JC5474 (*recC22*)	E_2	135	3	0.2	0	s

[a] Strains DR312, TR22, R-3 and R-7 were derived from W2252*thy*$^-$.
[b] Rescued cells were counted on trypsin plates after incubation with colicins for 30 min at 30 C.
[c] Sensitivity is expressed by s = sensitive, pr = partially resistant, and r = resistant.
[d] Minute colonies.

Absence of DNA degradation and inhibition of DNA synthesis by colicin E_2 in strain DR312. As expected of a mutant that exhibited trypsin-reversible death by colicin E_2, strain DR312 was found to suffer no DNA degradation by colicin E_2. When exponentially growing cells of strain DR312 containing DNA previously labeled with ^{14}C-thymine were treated with colicin E_2 (multiplicity = 120), no decrease in the radioactivity in the cold trichloroacetic acid-insoluble materials occurred, but cell division was stopped completely as described above (Fig. 2). This observation suggested that degradation of DNA into smaller fragments did not occur in the DR312 cells. Furthermore, no fragmentation of DNA into acid-insoluble pieces by colicin E_2 was observed to occur in strain DR312. The cells previously labeled with ^{14}C-thymine were incubated with colicin E_2 (multiplicity = 120) for 30 min at 30 C and then lysed by treatment with EDTA-lysozyme. The total lysate was then centrifuged through an alkaline sucrose density gradient. Distribution of radioactivity in the centrifuge tube showed that the average molecular size of DNA was the same as that of the control cells, a result that suggested that even single-strand breaks of DNA did not take place to any significant extent due to colicin E_2 (Fig. 3).

It was found that neither DNA degradation nor inhibition of DNA synthesis by colicin E_2 occurred in strain DR312. As shown in Fig. 4, incorporation of ^{14}C-thymine into the cold acid-insoluble materials in the presence of colicin E_2 proceeded at exactly the same rate as that of the control. Alkaline sucrose gradient sedimentation analysis showed that the average molecular size of DNA synthesized in the presence of colicin E_2 was also the same as that synthesized in the absence of the colicin.

Since cell division of strain DR312 stopped while DNA synthesis continued at the ordinary rate in the presence of colicin E_2, it was of interest to determine whether the segregation of newly synthesized DNA as discrete nuclear bodies distributed along the length of the filamentous cells at a constant interval occurred

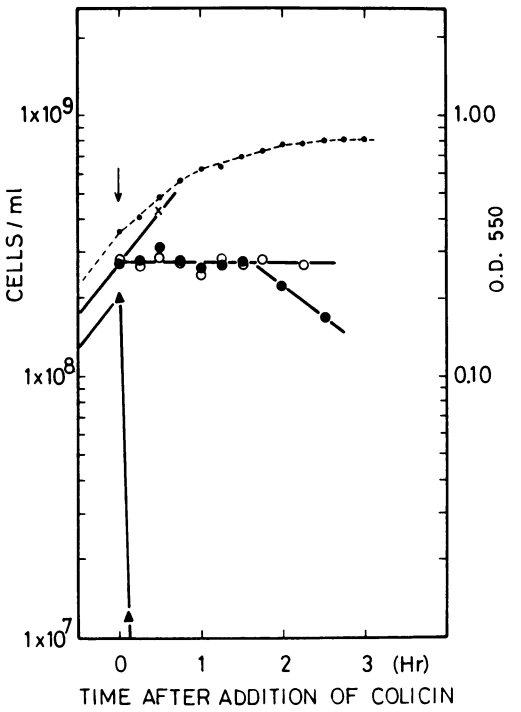

FIG. 1. *Effect of colicin E_2 on growth of strain DR312. At various times after the addition of colicin E_2 (at time 0), total cells (○) and viable cells (●) of strain DR312 were determined. Viable cells of strain DR312 without the colicin challenge (×) and viable cells of strain W2252thy⁻ after the addition of colicin (▲) were also measured. Optical density of the culture of strain DR312 after the colicin challenge was followed at 550 nm (dotted line).*

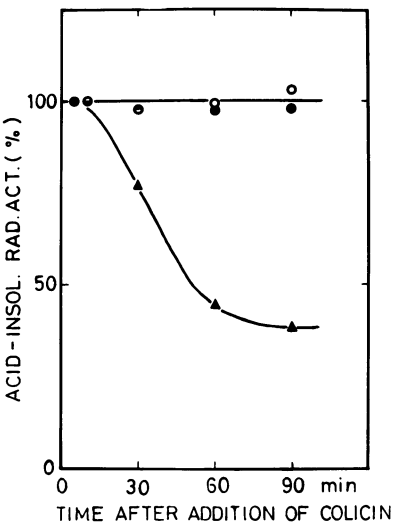

FIG. 2. *DNA degradation by colicin E_2 in strains W2252thy⁻ and DR312. After the addition of colicin E_2 to the DNA-labeled cells, radioactivity of the acid-insoluble materials in strain DR312 (●) and strain W2252thy⁻ (▲) was followed. Acid-insoluble radioactivity of DR312 without addition of colicin E_2 (○) was also measured.*

normally or whether the segregation was inhibited and a large concentrated nuclear region appeared at the center of the cells. Observation of the filamentous cells by the nuclear staining with Giemsa dye showed clearly that the former was the actual case (Fig. 5).

Absence of λ prophage induction and RNA degradation by colicin E_2 in strain DR312. It had been known that not only DNA degradation but also λ prophage induction (7) and degradation of ribosomal RNA (6) occurred in sensitive *E. coli* cells due to colicin E_2. Experiments were performed to observe whether these phenomena were induced by colicin E_2 in strain DR312 or not.

A λ-lysogenic DR312 was isolated from survivors from infection with λ phage, and its immunity was confirmed. This strain, DR312(λ), was grown in nutrient broth and treated with various amounts of colicin E_2 at 30 C for 40 min. Then 80 μg of trypsin per ml was added to digest the colicin, and, after ap-

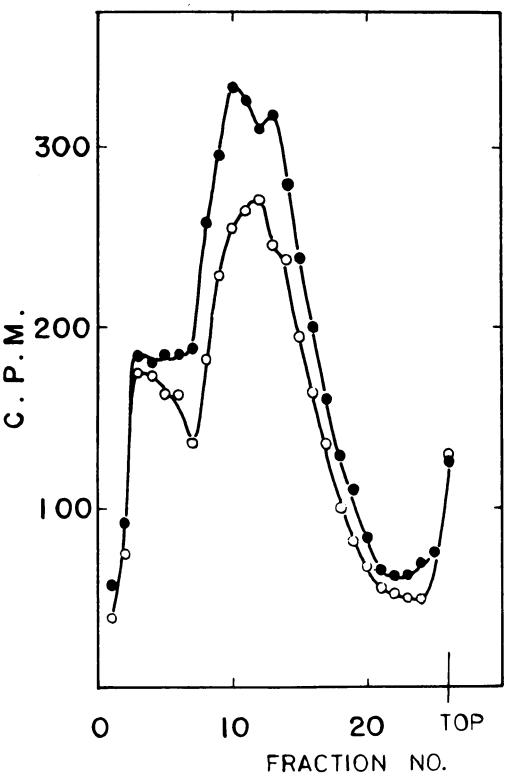

FIG. 3. *Alkaline sucrose gradient sedimentation profiles of DNA from cells of strain DR312 incubated with colicin E_2 for 0 min (○) and 30 min (●).*

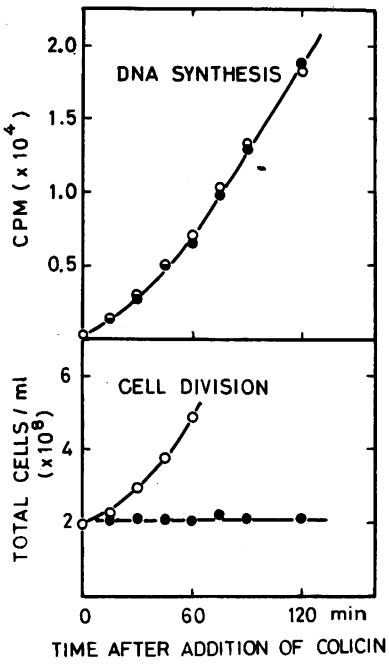

FIG. 4. *Effect of colicin E_2 on DNA synthesis and cell division of strain DR312. DNA synthesis was followed by measuring incorporation of ^{14}C-thymine, and cell division by measuring total cells. Measurements were performed with cultures of strain DR312 without colicin (○) and with colicin E_2 (●).*

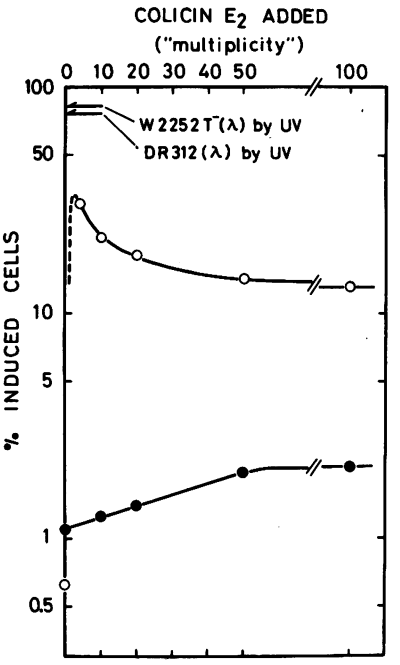

FIG. 6. *Induction of prophage λ of strains W2252thy⁻(λ) (○) and DR312(λ) (●) by various amounts of colicin E_2. Efficiency of induction by UV irradiation (at the dose of 99% killing of host cells), indicated by arrows, was 83.6% with strain W2252thy⁻(λ), and 75.6% with strain DR312(λ).*

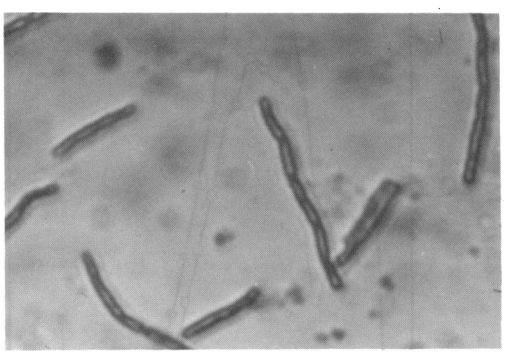

FIG. 5. *Nuclear staining of filamentous cells of strain DR312 incubated with colicin E_2 (multiplicity = 100) for 3 hr at 30 C.*

propriate dilution, the cells were plated with *E. coli* strain W2252*thy*⁻ as an indicator. Plaques were counted as the number of cells in which prophage was induced by the colicin challenge. As shown in Fig. 6, the fraction of cells exhibiting prophage induction by colicin E_2 was less than one-tenth of that of the parental λ-lysogen, W2252*thy*⁻(λ). On the other hand, the efficiency of inducing λ prophage by UV was the same in these two lysogens.

Degradation of ribosomal RNA induced by colicin E_2 occurred later and to a lesser extent than DNA degradation in the sensitive cells. Cells of strain DR312 in which RNA was previously labeled with ^{14}C-uracil were challenged with colicin E_2, and the decrease in radioactivity in the acid-insoluble materials was measured. However, no degradation of RNA upon the addition of colicin E_2 was observed in this strain. It was also observed that in strain DR312 synthesis of RNA measured by incorporation of ^{14}C-uracil and that of protein by incorporation of ^{14}C-methionine were not inhibited by the addition of colicin E_2.

Effect of colicin dosage on the inhibition of cell division of strain DR312. The experimental data presented above indicate that colicin E_2 caused specific inhibition of cell division without any other observable biochemical effects on strain DR312 at 30 C. Effect of the colicin dosage on the specific inhibition of cell division was examined. In experiments shown in Fig. 7, various amounts of colicin E_2 were added to the exponentially growing cultures of strain DR312 and incubated for about two

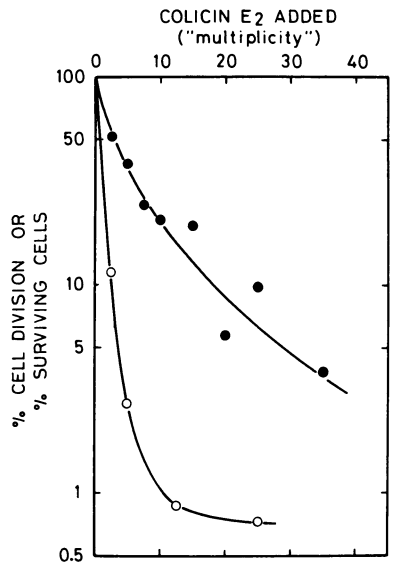

FIG. 7. *Inhibition of cell division of strain DR312 by various amounts of colicin E_2. Relative increase in viable cells of strain DR312 during the incubation with colicin E_2 (●) was measured as an index of cell division. Relative numbers of surviving cells of strain W2252thy⁻ (○) were also measured by use of trypsin plates.*

generations (100 min) at 30 C. The numbers of viable cells before and after incubation were measured by using the trypsin plates, and the per cent increases in viable cells at the various colicin dosages relative to the increase in the absence of colicin were calculated. The curve obtained by plotting these values against the colicin dosages showed that the inhibition of increase in viable cells (i.e., the inhibition of cell division) by colicin E_2 occurred at a very low dose without any cooperative effect.

Recovery of dividing ability due to trypsin. Incubation of strain DR312 with colicin E_2 resulted in long, filamentous cells with multiple nuclear bodies. After incubation for 60 min with the colicin, trypsin was added to such filamentous cells in liquid cultures and the increase in number of viable cells was followed. As shown in Fig. 8, cell division started after a lag period, and a considerable fraction of cells seemed to perform synchronous division initially. Thereafter, the number of viable cells continued to increase at the normal growth rate, and further stepwise increases could not be observed. The result suggested that the multinucleated filamentous cells formed by the colicin challenge did not divide into multiple progeny cells corresponding to the contents of the nuclear body at once but divided first into two viable cells.

UV and drug sensitivities of strain DR312. It had been suggested that UV sensitivity of microorganisms was related to a highly specific nuclease whose absence resulted in hypersensitivity to UV (14, 17). Since the absence of the DNA degradation by colicin E_2 in strain DR312 might be due to the absence of a specific deoxyribonuclease, the UV sensitivity of strain DR312 was compared to that of the parent. However, no difference of sensitivity between the two strains could be observed (Fig. 9). Two other radiation-sensitive mutants, with lesions in the *recB* and *recC* loci, were examined for their responses to the action of colicin E_2, but trypsin-reversible death by the colicin was not observed with these mutants (Table 1).

Several colicin-tolerant mutants had been reported to show hypersensitivity to various drugs owing to their defects in membrane structure (5, 11). But no difference in drug sensitivity was found between strain DR312 and the parent with the following chemicals: sodium deoxycholate, sodium dodecyl sulfate, sodium EDTA, methylene blue, novobiocin, bacitracin, vancomycin, erythromycin, and ristocetin.

DNA degradation by colicin E_2 in strain DR312 at various temperatures. All experiments described above were performed with cells of strain DR312 growing at 30 C, and no observable degradation nor breakage of DNA

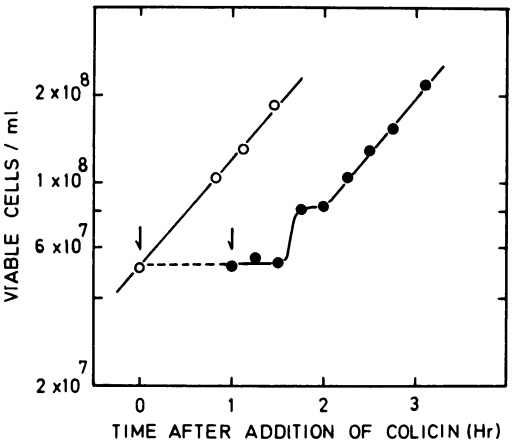

FIG. 8. *Recovery of dividing ability of the colicin E_2-treated strain DR312 in the presence of trypsin. Colicin E_2 (multiplicity = 100) was added to the exponentially growing culture of strain DR312 at 30 C at time 0, and after incubation for 1 hr, 40 μg of trypsin per ml was added. Viable cells were measured at various times by use of trypsin plates (●). Control experiment (○) without adding colicin and trypsin was carried out concomitantly.*

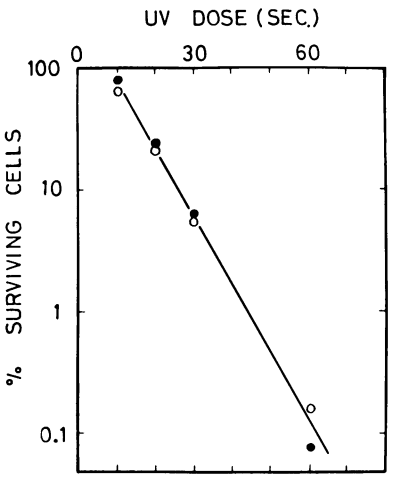

FIG. 9. *UV sensitivity of strains W2252thy⁻ (○) and DR312 (●).*

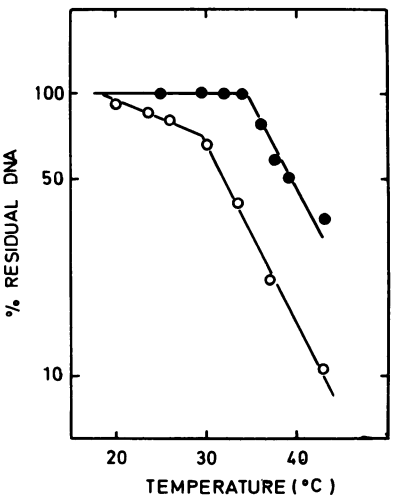

FIG. 10. *DNA degradation by colicin E_2 in strains W2252thy⁻ (○) and DR312 (●) at various temperatures. Residual radioactivity in the acid-insoluble materials of DNA-labeled cells after incubation with colicin E_2 (multiplicity = 100) for 45 min was measured.*

was brought about by colicin E_2. However, it was found that colicin E_2 could induce distinct degradation of DNA even in strain DR312 when the experiment was carried out at 37 C, and the trypsin reversibility of colony-forming ability was also decreased at this temperature. Colicin E_2-induced degradation of DNA into cold acid-soluble materials was measured at various temperatures with strain DR312 and the parent. Cells of strains DR312 and W2252*thy*⁻ were previously grown in the presence of ^{14}C-thymine at 30 C and then resuspended in fresh medium at various temperatures. After 20 min of incubation at the respective temperatures, colicin E_2 was added, and the DNA degradation during a 45-min incubation was measured. As shown in Fig. 10, the amount of DNA degraded in the 45-min period in strain W2252*thy*⁻ was temperature-dependent. However, between 18 and 30 C, the dependence was much less than it was between 30 and 45 C. In contrast, for strain DR312 between 18 and 34 C there was no DNA degradation, but the degradation between 34 and 45 C showed a temperature dependence essentially identical to that of DNA degradation in the parent strain.

DISCUSSION

In the mutant DR312, cell division was inhibited by colicin E_2, but none of the several other effects of colicin E_2 on the parent strain was observed, e.g., degradation of DNA into acid-soluble material, breakage of DNA strands detectable by alkaline sucrose gradient sedimentation, loss of the template activity of DNA to replicate, inhibition of the segregation of nuclear bodies, and the specific nucleolytic reaction to induce proliferation of prophage λ. These facts suggested that DNA in strain DR312 cells treated with colicin E_2 retained complete structural and functional integrity. Since a purified preparation of colicin E_2 was used in this study, the presence of contaminants inhibiting cell division at extremely low concentration might be excluded. Furthermore, the fact that several resistant and tolerant mutants showed distinct resistance to this preparation of colicin E_2 confirmed that the active agent inhibiting cell division was colicin E_2 itself. These results provided evidence that colicin E_2 had a specific inhibitory effect on cell division without any detectable degradation of DNA, and thus the inhibition of cell division was not a direct consequence of DNA degradation. The inhibition of cell division seems to be the primary action of colicin E_2 in causing DNA degradation; however, the possibility can not be excluded that these two phenomena are induced independently by colicin E_2. Whichever the actual case, the result presented in Fig. 7 indicated that only a small fraction of the colicin molecules (perhaps bound at special sites) inhibited division directly.

One possible explanation for the absence of DNA degradation in strain DR312 by colicin E_2 at 30 C might be that a specific deoxyribonuclease responsible for the reaction at the lower temperature in the parental, sensitive

cells (Fig. 10) was deficient in this strain. Since UV sensitivity and efficiency to produce phage λ by UV irradiation were not altered in the mutant and no relation was found with the recombination-deficient mutants tested, the functional importance of the assumed deoxyribonuclease in the normal cells, if present, can not yet be defined.

The fact that DNA degradation began to occur at a higher temperature was reminiscent of the sensitivity of the tolerant mutants *cetB* and *cetC* which were specifically tolerant for colicin E_2 at the lower temperature but sensitive at the higher temperature (9). In spite of this resemblance, growth of strain DR312 was completely suppressed permanently by colicin E_2 even at 23 C, an observation that indicated the essential difference of characters from *cet* mutants.

Trypsin-reversible death by colicin K had been assumed to indicate the location of the colicin molecules at the surface of the sensitive cells during its lethal action (10). Similar reversibility was observed only very temporarily with the colicin E_2-treated cells, thus making it doubtful that the location of colicin E_2 molecules was at the trypsin-accessible surface of cells throughout the action. The complete trypsin reversibility from the action of colicin E_2 observed in strain DR312 suggested that this colicin also remained at the surface during the course of its action.

Another trypsin-reversible mutant for colicin E_2, strain TR22, was not thoroughly investigated as it had a defect in its receptor structure. In this strain, DNA degradation still occurred, although to a lesser extent, while cell division also continued slowly in the presence of colicin E_2. Such a leaky property of strain TR22 may have resulted from the reversible adsorption of colicin molecules on the abnormal receptors.

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