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Isolation and Characterization of a Mutant Colicin E2

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Escherichia coli K-12 colicinogenic for Col E2 yielded a mutant, SK95, that carries a nonsense mutation in the colicin structural gene. A derivative of SK95 that carries an as yet unidentified suppressor mutation produces a colicin E2 that is temperature sensitive (TS). This mutant colicin kills sensitive cells at low temperature but not at high temperature; the colicin adsorbs to cells at high temperature but does not kill them unless the temperature is lowered. Unlike normal colicin E2, which adsorbs rapidly to cells, TS colicin E2 adsorbs slowly over a period of several hours. The biochemical target of colicin E2 is deoxyribonucleic acid (DNA). When acid solubilization of DNA was compared in cells treated with either TS or normal colicin E2, striking differences were observed. Cell killing and acid solubilization of DNA by colicin E2 were shown to be separable events under certain conditions. The results are discussed in relation to the mechanism of action of colicin E2.

The results presented in this paper concern the isolation of a mutant Col E2 factor with a nonsense mutation in the colicin structural gene and the characterization of the activity of a mutant colicin produced by a suppressed derivative of the strain with the mutant factor. The mutant colicin E2 exhibits an altered pattern of interaction with sensitive cells, which has implications for the mechanism of action of this colicin.

Adsorption of colicin E2 to sensitive cells initiates a succession of events that leads to more or less extensive enzymatic hydrolysis of deoxyribonucleic acid (DNA) to acid-soluble fragments and ultimately to cell death (6, 7). Colicin E2 is probably not itself a nuclease but may activate one or more nucleases or may interact with the DNA in such a way as to enhance the sensitivity of DNA to nuclease(s) (10). In any case, after colicin adsorption, first single- then double-strand breaks appear in the DNA (9) which are followed by nucleolytic degradation to acid-soluble fragments. There is circumstantial evidence for the involvement of endonuclease I in these events (1). Cell death cannot be reversed after the introduction of double-strand breaks (9). It will be shown in this paper that death can occur under conditions where there is little DNA degradation to acid-soluble fragments.

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A preliminary report of the isolation of this mutant has been published (C. K. Kennedy and S. H. Kleinman, Microbial Genet. Bull. 33:10, 1971).

MATERIALS AND METHODS

Organisms. The bacterial strains used are listed in Table 1.

Media. The compositions of LB broth and agar, OM agar, and soft nutrient agar have been published previously (5). Superbroth contains, per liter of medium: 32 g of tryptone, 20 g of yeast extract, 5 g of NaCl, and 5 ml 1 N NaOH in distilled water; 121 medium contains 1.2×10^{-1} M tris(hydroxymethyl)-aminomethane, 8×10^{-2} M NaCl, 2×10^{-2} M KCl, 2×10^{-2} M NH_4Cl, 3×10^{-3} M Na_2SO_4, 10^{-3} M $MgCl_2$, 2×10^{-4} M $CaCl_2$, 2×10^{-6} M $FeCl_3$ in distilled water with the *p*H adjusted to 7.5. For growing cells, 1.5 ml of 0.095 M KH_2PO_4 was added per 300 ml of medium.

Stab and lacunae tests. Stab and lacunae tests for colicin production have been described previously (5).

Transfer of Col factors. F-mediated transfer of Col E2 and of the mutant factor Col E2-95 to other strains was performed as previously described (5).

Mutagenesis and selection of mutants. M72(Col E2) was mutagenized with nitrosoguanidine as follows. An overnight culture grown in LB was diluted 1:50 into superbroth and grown to a density of about 10^9 cells per ml, and then 0.05 ml of culture was added to a tube containing 100 μg of nitrosoguanidine in 0.95 ml of 0.1 M citrate buffer, *p*H 5.5. The tube was shaken for 20 min at 37 C, and then the suspension was diluted 1:1,000 into LB broth. Sam-

TABLE 1. *Organisms*

Strain	Relevant markers	Source
Escherichia coli K-12		
M72(Col E2)	(F) (Col Ib) $lacZ_{am}$ trp_{am}	E. Signer
MSO	*pyrD, his, strA*	E. Signer
CR63	*supD*	C. Georgopoulos
LA347	*thr, leu, gal, lac, thi, supE*	S. E. Luria
K110	*supF*	C. Georgopoulos
E. coli B		
B/E	Resistant to E colicins	
B/I	Resistant to I colicins	

ples (1 ml) of the diluted mutagenized culture were distributed to individual tubes and incubated overnight at 37 C; the contents of the tubes were diluted to about 10^3 cells/ml, and 0.1 ml was plated on LB agar in 2.5 ml of soft nutrient agar. After incubation at 42 C for about 15 hr, *E. coli* B indicator cells were applied in soft agar. Colonies that appeared not to produce colicin were picked and tested further.

Titration of colicins. Appropriate dilutions of colicin were mixed with samples of log-phase sensitive cells of *Escherichia coli* B for a given amount of time. The cells were diluted and plated by spreading to determine survival (S). Untreated control cells were incubated for the same time interval in broth to determine S_0. Colicin titer was determined from the number of killing hits per cell, x, according to the relation $S/S_0 = e^{-x}$. When tubes and plates were to be incubated at 44 C, all materials were prewarmed at that temperature.

Solubilization of DNA by colicin E2. *E. coli* B/I was grown overnight at 37 C in 121 medium supplemented with thiamine (1 μg/ml), Casamino Acids (0.2%), glucose (0.2%), and KH_2PO_4 (10^{-2} M). Cells were diluted 1:100 into fresh medium and grown at 37 C to a density of about 5×10^7 to 6×10^7 cells per ml (Klett reading 18). ^{14}C-Thymidine (0.5 μCi/ml) and deoxyadenosine (250 μg/ml) were added, and the culture was shaken at 37 C to reach a density of 3×10^8 to 4×10^8 cells per ml (Klett reading 70). The culture was filtered, and the cells were washed several times with 121 medium and resuspended in 121 medium plus nonradioactive thymidine (200 μg/ml). Half of this culture was shaken at 30 C and the other half at 44 C for 20 min. Colicin was added and samples were removed at various times for determination of counts in both cold acid-soluble and -insoluble material.

Samples of 0.4 ml were pipetted into small centrifuge tubes containing 0.4 ml of cold 10% trichloroacetic acid. After 60 min the tubes were spun at 17,300 × *g* for 20 min. The supernatant fluid (0.4 ml) was pipetted into a scintillation vial containing 5 ml of Triton-toluene scintillation fluid and 0.2 ml of water. To measure counts remaining in acid-insoluble material, the precipitates were resuspended in 0.8 ml of 5% trichloroacetic acid and dissolved by heating in a boiling water bath for 30 min. A 0.4-ml sample was counted in Triton-toluene scintillation fluid on either a Beckman or Nuclear-Chicago scintillation counter.

RESULTS

Identification of nonsense mutation in M72(Col E2)-SK95. Mutant strain SK95 was isolated after mutagenesis of strain M72(Col E2). SK95 produces no colicin E2 when tested by the stab method or by plating for lacunae at any of several temperatures, but retains immunity to colicin E2. [M72(Col E2) and SK95 are also colicinogenic for Col Ib which was discovered only after isolation of mutant SK95 when small amounts of another colicin were detected by stab tests of SK95 incubated at 44 C for 2 to 3 days. Colicin Ib is produced in small amounts compared to colicin E2 and is usually undetected especially when *E. coli* B is used as indicator. The colicin Ib activity was identified by the following criteria: (i) SK95 shows cross-immunity with known col Ib strains; (ii) SK95 is sensitive to colicin Ia; and (iii) *E. coli* B strains which are resistant to the E colicins are sensitive to the SK95 colicin whereas B strains resistant to the I colicins are insensitive to the SK95 colicin. Every experiment characterizing the temperature-sensitive colicin E2 was performed with *E. coli* B/I as indicator.]

A series of genetic tests outlined in Fig. 1 and detailed in the following paragraphs revealed that the mutation in SK95 is suppressible by nonsense suppressors and is located on the Col E2 factor.

Amber mutant suppressing derivatives of SK95 were selected by plating cells on minimal lactose agar. The resulting colonies were assumed to be *sup* since M72 has the markers $lacZ_{am}$ and trp_{am}. The *sup* property of the derivatives was verified by testing with several phage T4 amber mutants. Some of the SK95 *sup* derivatives produced colicin E2. The size of the halo around stabs of these strains was smaller than that around M72(Col E2), indicating either that less colicin was produced or that the colicin was less active.

The Col E2 factor from SK95 (designated hereafter as Col E2-95) was transferred to

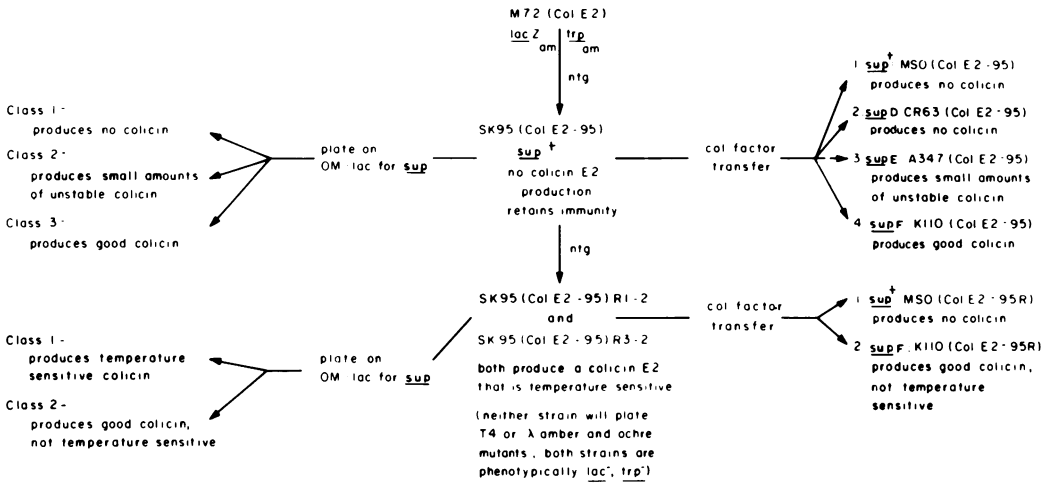

FIG. 1. *Genetics of mutant SK95. Abbreviations: ntg, nitrosoguanidine mutagenized; OM, minimal agar.*

other K-12 strains carrying different *sup* mutations. If no colicin was produced by a particular *sup* strain after mating, recipients were selected on the basis of having acquired immunity to colicin E2. CR63 *supD* (Col E2-95) produced no colicin. LA347 *supE* (Col E2-95) produced small amounts of an apparently unstable colicin E2; small halos were observed on stab plates incubated for 24 hr at 37 C, but no halos were detected on 48-hr stab plates. K110 *sup F* (Col E2-95) made nearly normal amounts of colicin E2. Strain MSO *sup*$^+$ (Col E2-95) produced no detectable colicin by stab testing.

At this stage it seemed likely that the mutation was on the Col E2-95 factor and was probably located in the colicin E2 structural gene, because some of the Col E2-95 suppressor strains produced small amounts of an unstable colicin E2.

To determine whether SK95 had a single nonsense mutation in the Col E2 factor, spontaneous colicin-producing revertants of SK95 were looked for. None were found among several hundred thousand colonies examined for colicin production, but the spontaneous reversion frequency may be too low. SK95 was then treated with nitrosoguanidine and plated by embedding cells in agar layers to score for colicin-producing colonies. Several colonies produced normal colicin. In addition, two independently mutagenized colonies, called SK95 R1-2 and SK95 R3-2, produced colicins that appeared to be temperature-sensitive (TS). To test this possibility, sets of plates were stabbed with the two strains, incubated at 30 C for 24 hr, and exposed to chloroform vapors for 45 min. One plate of each set was incubated at 44 C, and sensitive cells were then applied in soft agar; the plate was kept at 44 C while the indicator lawn grew. The other plate was kept at 30 C throughout application and growth of the indicator strain. A clear halo appeared around the stabs kept at 30 C, but none was apparent on the plates incubated at 44 C. It thus appeared that these mutants were producing a colicin E2 that was thermolabile or inactive at 44 C. This colicin was E2, because *E. coli* B/E was resistant to it whereas B/I was sensitive.

Filtrates of overnight cultures of the mutants R1-2 and R3-2 grown at 30 C were used in spot tests on lawns of sensitive indicator strain B/I on LB agar. The results of temperature shifts indicate that the inactive condition of the mutant colicin at 44 C is reversible after at least 1 hr of exposure to that temperature. Filtrates from cultures of the mutants mixed with filtrates from M72(Col E2) and then spotted on indicator cells after the temperature was raised did not cause loss of activity of the wild-type (WT) colicin E2. This eliminated the possibility that inactivation was due to production by the mutants of some substance that caused colicin inactivation at high temperature.

Although the inactive state of the mutant colicin appears to be reversible, no colicin activity could be found at 30 C from cultures of the mutants grown at 44 C either on a stab plate or in broth. Filtrates from broth cultures grown at 44 C failed to kill indicator cells at 30 C in spot tests. Experiments that further characterize the TS colicin will be presented in following sections.

It was desirable to determine whether strains SK95 R1-2 and SK95 R3-2 had under-

gone mutations from nonsense to missense in the colicin structural gene or whether the original nonsense mutation was being suppressed. The Col E2-95 factors were transferred to strains MSO *sup*$^+$ and K110 *supF*. MSO(Col E2-95 R1-2) and MSO(Col E2-95 R3-2) produced no colicin by stab testing at either temperature (30 or 44 C). K110(Col E2-95 R1-2) and K110(Col E2-95 R3-2) produced nearly normal-size zones of a colicin E2 whose activity was not affected by increasing the temperature to 44 C. It appears that the original suppressible mutation of SK95 is still present in R1-2 and R3-2.

Strains SK95 R1-2 or SK95 R3-2 did not support growth of several T4 and lambda phages with either amber or ochre mutations. Both strains also were still phenotypically Lac$^-$ and Trp$^-$. Some "true" nonsense suppressor derivatives of R1-2 and R3-2 (selected on minimal lactose agar) produced colicin E2 that is not TS. It is concluded that the suppressor mutations that have led to the production of strains R1-2 and R3-2 are not like the known nonsense suppressors. The nature of these mutations is still under investigation.

In summary, mutant SK95 derived from M72(Col E2) contains a nonsense mutation most probably in the colicin E2 structural gene. Two derivatives of SK95 contain unidentified suppressor mutations that cause them to produce a colicin E2 that is TS; the mutant colicin kills sensitive cells at low temperature but not at high temperature. Additionally, these mutants retain immunity at all temperatures as does SK95, indicating that active colicin is not responsible for immunity in colicinogenic strains.

Preparation of TS colicin E2. The TS colicin E2 was prepared in as gentle a manner as possible since its stability under various conditions was unknown. Strain SK95(Col E2-95) R3-2 was first made resistant to the E colicins to prevent adsorption of TS colicin to cells. Cells were grown overnight in LB broth at 30 C and then spun down; the supernatant fluid was sterilized by filtration. Higher titer preparations were obtained by adding 0.1 μg of mitomycin C per ml to the overnight culture. WT colicin E2 was prepared in an identical manner from strain M72(Col E2). Both WT and TS colicins were stored in small amounts in a freezer. *E. coli* B/I was the indicator strain used in all experiments described below.

An attempt to purify TS colicin by the procedure of Herschman and Helinski (3) failed because the TS colicin did not precipitate with ammonium sulfate as did similarly prepared normal colicin E2. Also the TS colicin decayed quite rapidly in the time required for purification.

Very little loss of recoverable activity occurred when TS colicin was kept as long as 48 hr at 44 C, indicating that although this colicin has low killing activity at high temperature, it is not subject to any substantial irreversible thermal inactivation.

As shown in Fig. 2, the TS colicin E2 shows single-hit killing action on sensitive cells after adsorption at either 30 or 44 C followed by plating at 30 C.

Adsorption of TS colicin E2. Preliminary experiments suggested that at 44 C the TS colicin E2 adsorbs to sensitive cells but does not kill them unless the temperature is lowered.

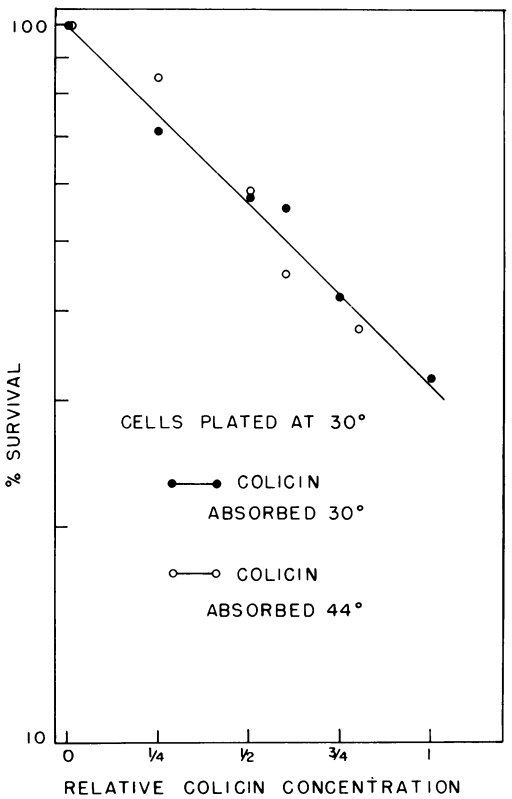

FIG. 2. *Single-hit bacterial killing by TS colicin E2. An E. coli B/I culture was grown in LB at 30 C to a density of about 4 × 10^8 cells per ml. Portions of culture were shifted to 44 C, and equal volumes of various dilutions of TS colicin E2 prewarmed to 44 C were added. Another portion of culture was kept at 30 C, and equal volumes of TS colicin dilutions were added. After an adsorption period of 30 min, the cells were diluted and plated by spreading on LB plates and incubated at 30 C. Control cells were mixed at the two temperatures with equal volumes of LB and similarly plated.*

The inactivity of TS colicin E2 at high temperatures is therefore not due to inability to be adsorbed.

The kinetics of adsorption of TS and normal colicin E2 was measured by mixing cells with colicin at both 44 and 30 C and plating samples from each incubation mixture at both 44 and 30 C. Figure 3A shows the adsorption kinetics of normal colicin E2 prepared from strain M72(Col E2). To give a basis for comparison, the concentration of WT colicin was adjusted to give a similar extent of killing as the TS colicin after 20 min of adsorption. As expected, adsorption of WT colicin was fast and complete within 10 min. Adsorption of TS colicin is shown in Fig. 3B (the TS colicin preparation used here is different from the one used for the experiment of Fig. 2). Even after 1 hr at the lower temperature, a significant fraction of the cells could be rescued by plating at the higher temperature. This was surprising since with WT colicin E2 the irreversible killing of cells has been shown to occur within 10 min after adsorption (9). It appears that the killing step occurs more slowly with TS than with WT colicin E2. The extent of killing by TS colicin adsorbed at 44 C for 1 hr and then plated at 30 C corresponds to a colicin concentration of more than 10 killing units of colicin per cell. The "44 → 44" survival curve indicates a concentration of two killing units per cell after 1 hr of adsorption. These numbers suggest either that 20% of the TS colicin molecules retain their ability to kill at high temperature or that the probability that an adsorbed TS colicin molecule will kill a cell is at least five times greater at 30 C than at 44 C. Similar experiments carried out for longer times showed adsorption of TS colicin continues for at least 2 to 3 hr.

The possibility was considered that the continuing loss of viable cells with time in TS colicin was due not to continued adsorption, but to a killing effect of the colicin that occurred

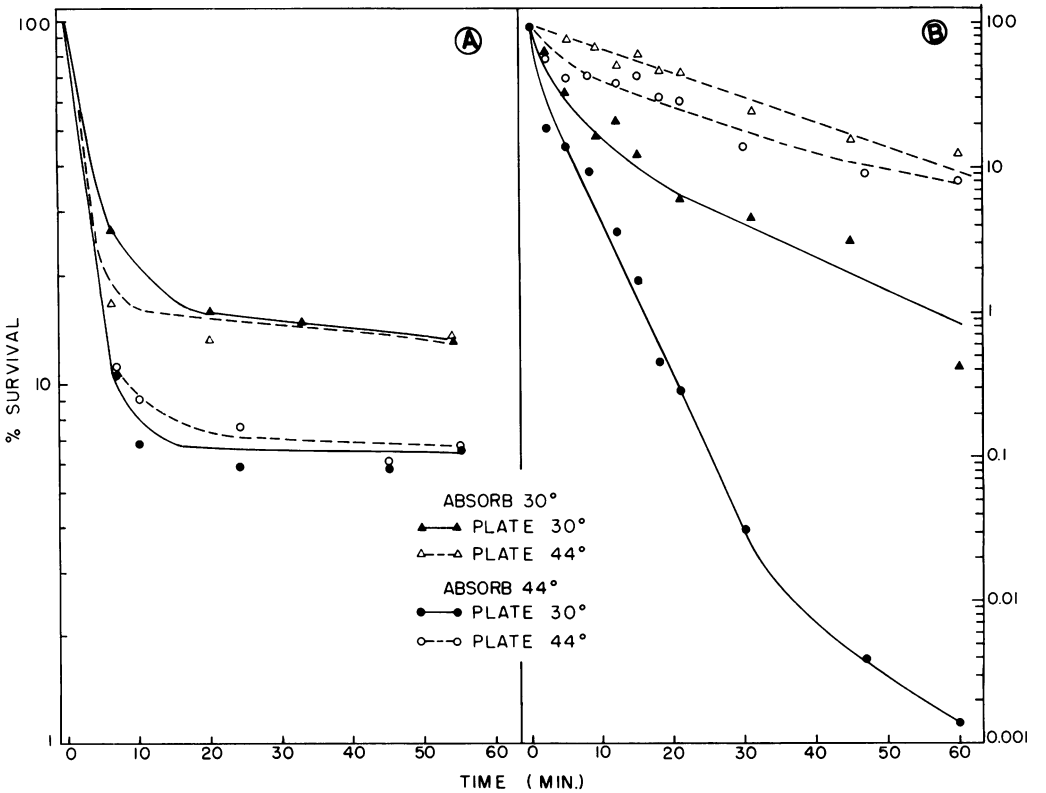

FIG. 3. *Kinetics of adsorption of WT and TS colicin E2. E coli B/I cells were grown to a density of about 4 × 10⁸ cells per ml in LB at 30 C. Portions of the culture were placed at 44 or 30 C, and equal volumes of either WT or TS colicin E2 (prewarmed to 30 or 44 C) were added. After various times, samples were diluted, plated by spreading on LB agar, and incubated at 30 or 44 C. When cells were treated with colicin at 44 C and plated at 44 C, dilution tubes, pipettes, and plates had been prewarmed at 44 C. Control cells were mixed with equal volumes of LB and similarly plated. A, WT colicin E2; B, TS colicin E2.*

in broth culture and stopped at the time of plating. This hypothesis was tested by allowing cells to adsorb TS colicin for a given length of time and then diluting the mixture 1:100 or 1:1,000 into colicin-free medium and following survival with time. For both WT and TS colicin E2 the fraction of surviving cells changed very little after the cells were removed from the colicin. Thus the continued killing by TS colicin with time in growth medium is a reflection of the continued adsorption of colicin to cells.

TS colicin adsorbed at 44 C allows cells to divide. In addition to causing breakdown of cellular DNA, colicin E2 inhibits cell division (4, 8). TS colicin adsorbed at 44 C does not cause this inhibition. Figure 4 shows that when cells are exposed to a low multiplicity of TS colicin E2 at 44 C and are plated at intervals at 44 C, some cells are initially killed while others continue to divide at the rate of untreated cells. The dividing cells had adsorbed colicin since they were killed when plated at 30 C.

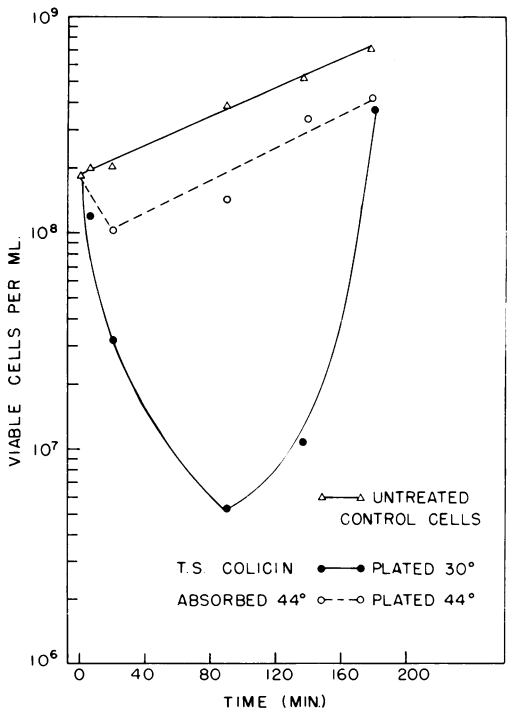

FIG. 4. *Effect of TS colicin on cell division at high temperature. An E. coli B/I culture was grown in LB at 44 C to a density of about 4 × 10^8 cells per ml. An equal volume of TS colicin prewarmed to 44 C was added, and after various times cells were removed and plated at 30 and 44 C. Untreated control cells were mixed with an equal volume of LB broth.*

Solubilization of DNA by TS colicin E2. The biochemical "target" of colicin E2 action is DNA (6). The primary lesion is probably a single-strand nick caused by some endonuclease activity; then double-strand breaks appear in the DNA and are followed by breakdown of the DNA into acid-soluble fragments (9). Even under conditions of nearly complete killing ($S/S_0 < 1\%$) about 20 to 40% of the cellular DNA remains acid-insoluble; this value varies among strains (4, 9; L. Saxe, *personal communication*).

Figure 5 presents the results of measurements of acid solubilization of prelabeled cellular DNA in *E. coli* B/I by WT and TS colicin E2. It can be seen that at 30 C TS colicin caused less DNA solubilization than did WT colicin E2, even though the killing multiplicity of TS colicin was higher. For both colicins solubilization was complete by 100 min. After treatment of cells with WT colicin, the DNA was degraded to a greater extent at 44 than at 30 C, making even more significant the low level of solubilization caused by TS colicin E2 at 44 C. The background level of 20% acid-soluble thymidine counts is constant and reproducible. This is felt to be a property of the *E. coli* B/I strain since K-12 strains consistently have much less background under the same conditions.

Temperature shift experiments were then performed to determine whether DNA degradation would continue or stop under conditions that inhibit the killing action of TS colicin E2. Colicin was added to cells (prelabeled with ^{14}C-thymidine) at either 30 or 44 C. At intervals, a sample was removed and incubated at the other temperature (30 or 44 C), and the extent of DNA solubilization was followed with time. The results are shown in Fig. 6. DNA degradation was inhibited if the temperature was shifted from 30 C to 44 C at early times; shifting at later and later times, however, stopped solubilization less effectively; by 80 min, raising the temperature actually enhanced the extent of DNA degradation, as it also did with WT colicin E2 (Fig. 6B).

When TS colicin was adsorbed at 44 C and the temperature was shifted to 30 C at various times, it was expected that solubilization of DNA would begin at an increased rate shortly after the temperature change since TS colicin kills the cells at low temperature. The expected result was not obtained, however: the experiment presented in Fig. 6C shows that little solubilization of the DNA occurred after a shift from 44 to 30 C. Even after 3 hr at the lower temperature (a condition that allows TS

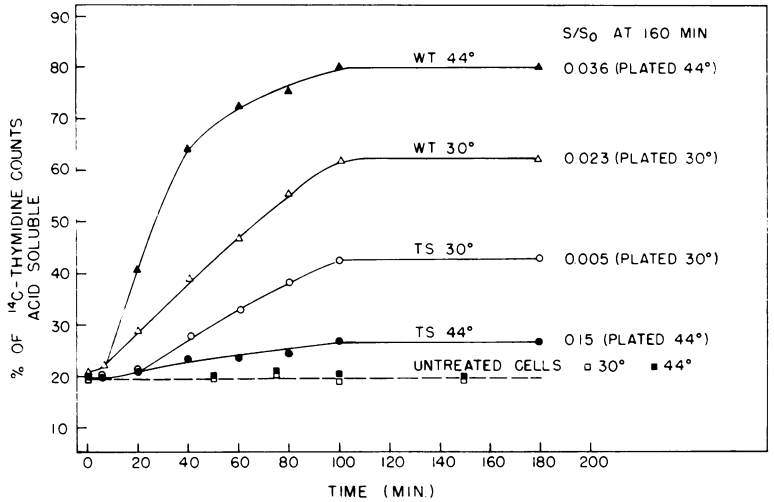

FIG. 5. *Effect of WT and TS colicin E2 on acid solubilization of DNA at 30 C and 44 C. An E. coli B/I culture was grown at 37 C to a density of about 6 × 10⁷ cells per ml in 121 minimal medium. ¹⁴C-thymidine (0.5 μCi/ml) and deoxyadenosine (250 μg/ml) were added and the culture was shaken at 37 C to reach a density of 3 × 10⁸ to 4 × 10⁸ cells per ml. Cells were filtered, washed, and resuspended in medium containing non-radioactive thymidine (200 μg/ml). Half the culture was shaken at 30 C and the other half at 44 C for 20 min. Equal volumes of TS or WT colicin were then added, and samples were removed at various times for determination of counts in both cold acid-soluble and insoluble material as described in Materials and Methods.*

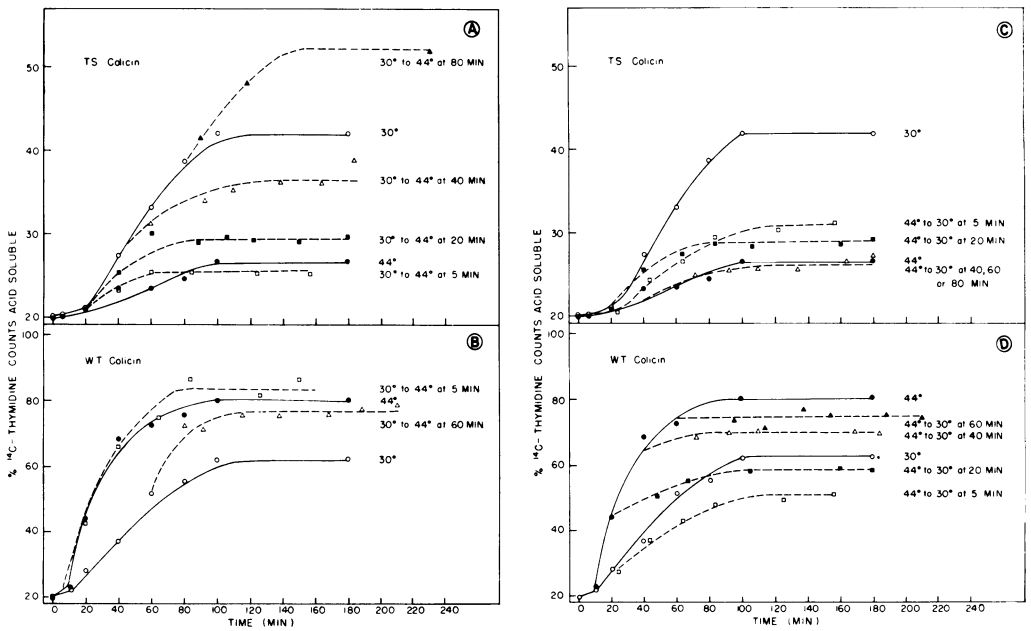

FIG. 6. *Effect of temperature shifts on acid solubilization of DNA by WT and TS colicin E2. The experiment was performed as described in Fig. 5. (Since the experiment was done at the same time as that shown in Fig. 5, the control values, without temperature shift, are the same as in Fig. 5.) After various times, a portion of each cells-plus-colicin mixture was shifted to the other temperature, and solubilization of DNA was monitored as described previously. Survival values measured at 240 min by platings at both 30 and 44 C were all <0.02 (tests on TS colicin E2 only). A, TS colicin, 30 C to 44 C; B, WT colicin, 30 C to 44 C; C, TS colicin, 44 C to 30 C; D, WT colicin, 44 C to 30 C.*

colicin to kill the cells), the extent of degradation was quite low compared to that observed when the temperature was maintained at 30 C throughout. Figure 6D shows that, with WT colicin E2, shifting the temperature from 44 to 30 C has a slightly inhibitory effect on DNA breakdown.

Both temperature shift experiments with TS colicin E2 suggest that sometime after adsorption an irreversible step occurs that causes a fixed extent of solubilization, which cannot be altered by changing the temperature. This is the expected result in going from 30 to 44 C since, after a certain time, thermal inactivation of the colicin should not affect DNA breakdown that has already been initiated. Since a shift from 44 to 30 C apparently allows reactivation of TS colicin, in the sense that cells are killed, it is surprising that no additional solubilization occurs when the temperature is shifted down. This result shows that killing of cells by colicin E2 is at least to some extent independent of solubilization of the DNA.

DISCUSSION

The suppressible nonsense mutation in strain SK95 is probably located in the colicin E2 structural gene, which would verify that the extrachromosomal Col E2 factor includes the genetic information for the colicin E2 protein. Evidently the activity of this protein can be altered by mutation. The "revertant" strains R1-2 and R3-2 derived from SK95 by nitrosoguanidine mutagenesis produce a TS colicin E2. These two strains appear to have an additional mutation that suppresses the original SK95 mutation. The Col E2 factors from R1-2 and R3-2 apparently still carry the nonsense mutation of the Col E2-95 factor in the colicin E2 structural gene. The suppressor mutations in R1-2 and R3-2 do not suppress any of several nonsense mutations in either the *E. coli* cells or in infecting lambda or T4 phage. The nature of these suppressors requires further investigation.

The colicin E2 produced by strains R1-2 and R3-2 kills sensitive bacteria effectively at 30 C but much less at 44 C. This TS mutant colicin does adsorb to bacteria at the higher temperature and then kills them if the temperature is lowered to 30 C. A certain amount of killing occurs also at the higher temperature.

It is difficult to characterize a preparation of the TS colicin in terms of concentration of killing units since adsorption of this colicin to sensitive cells at 30 C is a slow process, and although faster at 44 C it is still quite slow. Adsorption of WT colicin E2 under standard conditions is usually complete in a few minutes at either temperature. Thus, in addition to losing full activity at 44 C, the mutant colicin E2 has been altered in ability to adsorb to sensitive cells.

It is unclear whether the degradation of DNA to acid-soluble fragments that occurs in cells treated with colicin E2 is a primary or secondary consequence of E2 action. After single-strand and double-strand breaks are introduced in the DNA, exonucleases (not necessarily activated by colicin E2) would be expected to cause degradation. This hypothesis is somewhat supported by the observation that *recB* or *recC* mutants, which lack an exonuclease activity, show less acid solubilization of DNA after treatment with colicin E2 (R. R. Hull and P. Reeves, *submitted for publication;* L. Saxe, *personal communication*).

The fact that TS colicin E2 causes less degradation of DNA to acid-soluble fragments than WT colicin E2 under conditions in which cells are killed to a similar extent can be explained in two ways. First, TS colicin E2 may act slowly on cells so that more repair of DNA breaks can take place than after WT colicin E2 action. Breaks may be introduced at a slow rate, which would result in an increased probability of their being repaired so that less exonucleolytic breakdown would occur. Slow action of TS colicin E2 (apart from slow adsorption) is suggested by the fact that a significant fraction of cells treated with TS colicin E2 at low temperature can still be rescued by raising the temperature (or by treating the cells with trypsin [*unpublished data*]) 30 to 60 min after adsorption. Cells treated with WT colicin E2 are not affected by temperature, and killing of cells is reversible by treatment with trypsin for only a few minutes (9; *unpublished data*).

Second, exonucleolytic activity may be a direct consequence of colicin E2 adsorption, possibly because of activation of cellular exonucleases. In this case, alteration of the molecule by mutation may result in reduced nuclease activation.

Increased DNA solubilization by WT colicin E2 at high temperature may be correlated with the fact that the nicks that occur spontaneously in the DNA of *E. coli* are more abundant when a culture is shifted from low to high temperatures (2). The fact that the increased DNA solubilization at 44 C occurs in cells treated with WT colicin E2 but not in control cells may indicate either an increased rate of DNA breaks produced by E2 or activation of exonucleases by E2. The small extent of degra-

dation that occurs in cells treated at high temperature with TS colicin E2 might be due in part to an increase in spontaneous breaks at high temperature.

It is clear from the present results that cell killing can be dissociated to some extent from acid solubilization of DNA, and this raises the possibility that killing of cells by colicin E2 can occur independently of its effect on DNA. It would be desirable to look at the appearance of single- and double-strand breaks in DNA after treatment with TS colicin in order to correlate their occurrence with cell death. Since TS colicin appears to act more slowly on sensitive cells, it may make it possible to determine precisely the sequential relation of solubilization of DNA to the occurrence of single- and double-strand breaks.

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