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The BtuB Group Col Plasmids and Homology Between the Colicins They Encode

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Colicins A, E1, E2, E3, E4, E5, E6, and E7 exhibited reduced activity against BtuB mutants of *Escherichia coli* K-12 and also against wild-type cells in the presence of vitamin B12. Plasmids encoding representatives of these colicins were specifically immune to high levels of the homologous colicin. Col$^+$ cells grown in media containing mitomycin C accumulated large amounts of colicin polypeptide. ColE2$^+$, ColE3$^+$, ColE4$^+$, ColE5$^+$, and ColE6$^+$ cultures also synthesized large amounts of second, lower-molecular-weight protein under these conditions. Colicins E2 through E7, but not A or E1, reacted with antiserum raised against purified colicin E3. Colicins E2 and E7 induced synthesis of β-galactosidase encoded by *lacZ* under the control of the colicin Ib gene promotor on a derivative of Col plasmid ColIb.P9. This promotor is usually active only when the cells are treated with agents which damage DNA or block replication. Plasmids encoding various mutant forms of colicin E3 (M. Mock and M. Schwartz, J. Bacteriol. 142:384–390, 1980) recombined with ColE2, ColE4, ColE5, or ColE6 plasmids at a frequency of 10^{-4} per cell to produce a colicin active against ColE2$^+$, E4$^+$, E5$^+$, or E6$^+$ cells. ColE5 and ColE6 plasmids recombined with ColE3 plasmids bearing mutations affecting colicin E3 receptor recognition, envelope penetration, and catalytic activities. ColE2 and ColE4 plasmids recombined only with ColE3 plasmids bearing mutations affecting receptor recognition and envelope penetration. Recombinants between mutant ColE3 plasmids and ColA, ColE1, or ColE7 plasmids were not detected. We propose the designation BtuB group for the colicins described here, and we divide the group into two classes comprising colicins A and E1, which act on the cytoplasmic membrane, and the related colicins E2 through E7, which have known or putative nuclease activities.

Certain colicins can be grouped on the basis of their reduced activity against *Escherichia coli* K-12 mutants lacking the outer membrane BtuB protein, the receptor for vitamin B12 and for bacteriophage BF23 (12, 18, 18a, 31). These colicins can be divided into different types by the absence of substantial colicin cross-immunity between the producing strains (4, 18, 18a, 31), and four distinct immunity types (A, E1, E2, and E3) have been extensively characterized. Colicins A and E1 form small channels in artificial lipid bilayer systems (11, 33, 38; F. Pattus, personal communication). It has been suggested that channel formation enables these colicins to dissipate the energy potential across the cytoplasmic membrane of treated cells, resulting in reduced ability to accumulate certain solutes by active transport (16), but other explanations also seem possible (13). The colicin recently described by Cavard and Lazdunski and named E4 by them (3) and K by others (17) is indistinguishable from authentic colicin A on the basis of activity spectra against a range of colicin-insensitive mutants of *E. coli* K-12, by the high level of immunity to the Cavard and Lazdunski colicin exhibited by ColA$^+$ *E. coli* K-12 derivatives, and by the serological cross-reactivity of the two colicins (3a, 17, 18a). Colicins A and E1 can be distinguished from each other by differences in chemical properties and activity spectra (3, 4). Colicin E2 has deoxyribonucleolytic activity, and colicin E3 has ribonucleolytic activity on 16S ribosomes (2, 6, 32). Colicins E2 and E3 exhibit substantial chemical and serological homology, and it is probable that these colicins differ markedly only in their catalytic domains and the regions to which the homologous colicin immunity proteins bind (9, 23, 35, 41).

Horak (10) and Males and Stocker (18, 18a) have screened a number of bacterial strains

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producing colicins which were inactive against BtuB mutants of *E. coli* K-12 and have identified four apparently new colicin immunity types (designated colicins E4, E5, E6, and E7 by Males and Stocker). Our own characterization of *E. coli* K-12 mutants selected as insensitive to each of these colicins indicated that they could be distinguished from each other and from colicins A, E1, E2, and E3 (unpublished data). We therefore wished to determine the extent of homology, if any, between representatives of the eight colicin types. We first constructed a set of isogenic *E. coli* K-12 derivatives each producing one of the eight colicins and used these strains to characterize the Col plasmids. The colicins were compared with respect to their modes of action and adsorption to sensitive cells. Results of these experiments indicated that the newly identified colicins were related to colicins E2 and E3. This relationship was confirmed by serological methods and by genetic studies in which we sought to recombine plasmids carrying mutated colicin E3 genes (20, 22) with plasmids carrying wild-type genes encoding other colicins to produce active colicin E3.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strains carrying each of the Col plasmids were constructed by transformation of *E. coli* K-12 strain W3110 or its sodium nalidixate-resistant derivative (BZB1011) by using plasmid DNA extracted from the prototype colicinogenic strains (Table 1) with selection for colicin immunity in the recipients. Several intermediate transformation steps with low concentrations of plasmid DNA and initially with colicin I-resistant recipients (for ColE4.CT9 and ColE6.CT14 transformation) were used to obtain derivatives carrying only one plasmid type. Each of the Col^+ derivatives produced only one colicin as judged by activity spectra against a range of colicin-insensitive *E. coli* K-12 mutants and was specifically immune (insensitive) to high doses of the colicin it produced (Table 1). Col^+ derivatives were made $RecA^-$ as described previously (24). Col^+ transformants of strains 1100 and pp78 (1100 *cya crp*) were used to study the effects of catabolite repression on colicin production.

The presence of the Col plasmids did not restrict development of bacteriophages T2, T3, T4, T5, T6, T7, P1, BF23, Mu-1, or λ, although strains carrying other extrachromosomal elements including Col plasmids are known to restrict development of some of these bacteriophages (7). The Col^+ strains remained fully sensitive to kanamycin, chloramphenicol, sulfazurole, tetracycline, streptomycin, erythromycin, novobiocin, oleandomycin, ampicillin, gentamycin, and rifampicin in antibiotic sensitivity disk tests. Strains carrying mutations in the colicin E3 structural gene (*ceaC*, colicin E activity) were isolated previously (20–22). The colicin indicator strains were W3110 and its nalidixate- or streptomycin-resistant derivatives, unless otherwise stated.

Media, growth conditions, and tests for colicin production, colicin receptor activity, and colicin immunity were as described elsewhere (9, 14, 22, 27).

TABLE 1. Colicinogenic strains and Col plasmids[a]

Prototype strain	Colicins produced	W3110 derivative	Col plasmid	Plasmid size[b] (base pairs)	Level of homologous colicin immunity[c]	Molecular weight of putative colicin[d]
CA31	A	BZB2101	ColA.CA31	4,200	$>10^3$	58,000
K53	E1	BZB2104	ColE1.K53	5,400	300	54,000
KH293	E2	BZB2125	ColE2.P9	6,000	$>10^4$	67,000
BZB2006	E3	BZB2106	ColE3.CA38	5,800	$>10^5$	67,000
316BM	E4, I	BZB2107	ColE4.CT9	6,000	$>10^4$	66,000
271BM	E5	BZB2108	ColE5.099	5,800	$>10^5$	67,000
318BM	E6, I	BZB2109	ColE6.CT14	11,000	$>10^5$	67,000
245BM	E7	BZB2110	ColE7.K317	6,100	$>10^5$	67,000

[a] Strain CA31 is *Escherichia freundii* (*Citrobacter freundii*), strain K53 is *E. coli*, and all other strains are derivatives of *E. coli* K-12. Plasmid nomenclature follows the recommendations of Reeves (31) and indicates the colicin produced and the strain from which the plasmid was originally derived. Details of the origins of these plasmids can be found in references 18, 18a, and 31.

[b] Plasmid sizes were determined by agarose gel electrophoresis of plasmid fragments obtained by cleavage with restriction endonucleases *Eco*RI, *Hin*dIII, *Sal*I, *Bam*HI, *Ava*I, *Pvu*II, *Bgl*II, and *Sac*I and comparison with the *Hin*dIII and *Eco*RI restriction fragments of bacteriophage λ DNA. In each case, the average of three determinations with three different enzymes is presented. Restriction fragments of less than 200 base pairs were not resolved, and the figures given may therefore be an underestimate of the actual sizes.

[c] The level of colicin immunity was determined by an endpoint spot dilution assay with lawns seeded with 10^6 cells of each strain. The figures are given as dilutions of colicin required to give a clear inhibition zone relative to strain W3110.

[d] The molecular weights of the putative colicin polypeptides were determined by polyacrylamide gel electrophoresis in the presence of SDS (see example in Fig. 1) with molecular weight standards. The polypeptides behaved in a manner characteristic of soluble proteins in the presence of SDS in Ferguson analyses (29), and there is no reason to suspect that their molecular weights determined by this method are incorrect.

The ability of vitamin B12 to prevent colicin activity was assayed by the method used previously to demonstrate inhibition of colicins by enterochelin, except that tryptone broth was used in place of minimal medium (25). Partially purified inactive colicin E3 from mitomycin C-treated cells carrying a plasmid bearing the *ceaC1* mutation (22) was used in the same assay in place of vitamin B12 to assess its ability to protect cells against other, wild-type colicins. The lacuna assay was as used by Hardy et al. (8). A lacuna was assumed to represent a zone in which sensitive bacteria were killed by colicin released from a single Col^+ cell. Procedures for the isolation and purification of closed-circle plasmid DNA by alkaline sodium dodecyl sulfate (SDS) extraction (1) and elution from agarose gels with hydroxylapatite (36) for the use of restriction endonucleases, agarose gel electrophoresis, and transformation (5) and for R64*drd-11*-mediated conjugal transfer of Col plasmids (26) were as described previously.

Serological analysis of colicins by double-diffusion immunoprecipitation with antiserum raised against purified colicin E3 was as used previously (21). In an alternative immune precipitation assay, crude colicin from the supernatant fraction of a French pressure cell lysate of mitomycin C-treated cultures (27) was diluted to 1 mg of protein per ml (determined by a modified Lowry reaction [29]) in 10 mM Tris buffer (pH 7.1 with HCl) and reacted with dilutions of the antiserum for 30 min at 37°C. The immune precipitates, when present, were pelleted by centrifugation at 20,000 × *g* for 3 min and washed twice in Tris buffer. The precipitates were then dissolved in SDS sample buffer (29) and examined by SDS-acrylamide gel electrophoresis with the Tris-glycine buffer system with 11% acrylamide separation gels (29). In the colicin neutralization assay, crude colicin was diluted so that a 1-ml sample was sufficient to kill 95% of 10^8 cells in 5 min at 37°C. Diluted colicin in 1-ml lots was mixed with 1 ml of doubling dilutions of the antiserum at 37°C for 30 min. Indicator cells were then added to give a concentration of 5×10^7 per ml, and incubation was continued for 5 min before the cells were plated to count survivors. The extrapolated endpoint titer of the antiserum was that dilution which gave more than 90% cell survival.

Proteins whose production was stimulated after mitomycin C treatment of Col^+ cells were identified by SDS-acrylamide gel electrophoresis of whole cells or of material extracted with Triton X-100 in the presence of 0.1 mM EDTA (27, 29). The procedures used to label these proteins with ^{14}C-amino acids were as before (20). Partially purified colicins were prepared from French pressure cell lysates of mitomycin C-treated Col^+ cultures by ammonium sulfate precipitation and gel filtration on Sephadex G200 under nondenaturing conditions (26).

The effects of colicins on amino acid uptake were determined by using cells grown at 37°C to the early exponential phase in tryptone broth. The cells were washed twice and suspended to a concentration of 3×10^8/ml in minimal medium (19) containing 100 μg of chloramphenicol per ml. Colicin was added, and the cells were incubated at 37°C for 5 min before the addition of [^{14}C]proline (10 μM, 170 μCi/mmol). Other procedures were as used previously (28).

The effects of colicins on protein synthesis were determined by using cells growing exponentially in minimal medium. Colicin and ^{14}C-amino acids (0.5 μCi/ml) were added as above, and 0.6-ml samples were withdrawn at time intervals. Proteins were precipitated with 6% cold trichloroacetic acid and then filtered and counted as above. The effects of colicins on synthesis of preinduced β-galactosidase were determined by using cells growing exponentially in tryptone broth to which 2 mM isopropyl-β-D-thiogalactopyranoside was added 10 min before the colicin. Samples withdrawn at intervals after colicin addition were assayed for β-galactosidase (19). The effects of colicins on transcription of the colicin Ib gene were determined by using strain CSH55 Δ(*lac-pro*) harboring pAPBZ153, a derivative of ColIb.P9 in which the colicin Ib gene (*ciaB*) had been fused to the structural gene for β-galactosidase (*lacZ*; 24). Colicin was added to cells growing exponentially in tryptone broth, and cells were withdrawn at intervals and assayed for β-galactosidase (19). β-Galactosidase is usually only synthesized by this strain after treatment with agents which damage DNA or inhibit DNA replication (24).

The procedure used to estimate plasmid copy numbers was as follows. Cultures of the Col^+ cells and W3110 derivatives bearing plasmids pBR322, pSC101, or pDF42 (copy numbers 30 to 50, 5 to 8, and 1 or 2, respectively; S. Iida, personal communication) were grown to the same cell density and mixed before the plasmids were extracted (1). The plasmids (95% closed circle form) were then resolved by agarose gel electrophoresis (5), stained with ethidium bromide, and photographed with Polaroid 665 film. An integrated densitometry scan of the film negative was used to compare the recovery of the two plasmids and, with the known sizes of the plasmids, to calculate their copy number. This procedure gives only an approximation of the copy number.

Recombination analysis. Strains carrying two Col plasmids were constructed by R64-mediated transfer of mutant ColE3.CA38 plasmids into W3110 derivatives which already produced a colicin of a different type. Two milliliters of melted soft agar seeded with approximately 5×10^3 cells of the transconjugants was poured onto a plate of L agar. After it had set, a second layer of 4 ml of sterile soft agar was added, and the plates were incubated for 18 h at 37°C and then overlaid with soft agar containing 10^7 cells of a suitable indicator strain which was immune to the wild-type colicin produced by the recipient. In certain cases (see below), inhibition zones appeared at a frequency of approximately 10^{-4} per transconjugant (compared with a reversion frequency of approximately 10^{-7} for the *ceaC* mutations). These inhibition zones were assumed to be formed as a result of recombination between the mutant *ceaC* genes and the wild-type colicin E genes present on the coresident plasmids. Colonies carrying putative recombinant Col plasmids were reisolated free of indicator and nonproducing cells and retested for colicin production. Transconjugants carrying putative recombinant Col plasmids produced normal levels of both the wild-type and the recombinant colicin E3, as judged by colicin zone widths produced by colonies growing on LA plates and by the titers of colicins obtained from French pressure cell lysates (27) of uninduced cultures when tested with heteroimmune indicators. A similar recombination assay to that described here has been used by others to examine the relationships among chromo-

somal *trp* operon genes in various strains of *Enterobacteriaceae* (34).

RESULTS

Col plasmids and encoded proteins. The colicinogenic W3110 derivatives (Table 1) each carried a small plasmid present at an estimated 10 to 20 copies per cell. Strains carrying these Col plasmids were unable to transfer colicin immunity by conjugation unless a known conjugative plasmid (F'*lac* or R64*drd*) was also present in the donor (Col^+) cells.

Col^+ cultures contained a higher number of colicin-producing cells and synthesized more colicin after treatment with 500 ng of mitomycin C per ml (Table 2) or 20 U of bleomycin per ml (data not shown). Mitomycin C-treated cultures accumulated large amounts of a protein which comigrated with homologous purified colicin in SDS-polyacrylamide gels (Fig. 1). The molecular weights of these proteins corresponded well with the published colicin molecular weight estimates for the $ColA^+$, $ColE1^+$, $ColE2^+$, and $ColE3^+$ cultures (Table 1) (3, 15). The apparent molecular weights for the proteins produced by the $ColE4^+$, $ColE5^+$, $ColE6^+$, and $ColE7^+$ cultures were similar to those of colicins E2 and E3 (Table 1). No other plasmid-encoded, mitomycin-stimulated proteins were detected in $ColA^+$, $ColE1^+$, or $ColE7^+$ cultures, but additional, low-molecular-weight polypeptides were detected in mitomycin C-treated cultures of the $ColE2^+$ through $ColE6^+$ strains (Fig. 1B). These polypeptides presumably correspond to the colicin immunity proteins (37).

Synthesis of all eight colicins was reduced when the cells were grown in the presence of 0.5% glucose or when the strains were constitutively catabolite repressed due to mutations in the *cya* and *crp* genes (Table 2). The effect of glucose was reduced when the growth medium also contained 1 mM cyclic AMP (Table 2). Colicin synthesis was also lower in strains carrying the *recA56* mutation (Table 2). These results indicate the overall similarity of the mechanisms controlling production of the eight colicins.

Crude extracts prepared from each of the Col^+ strains were used for serological analysis with antiserum raised against purified colicin E3 (21). In immunodiffusion assays, the colicin E2, E4, E5, E6, and E7 preparations cross-reacted with this antiserum and displayed a reaction of partial homology with colicin E3 (Fig. 2). Colicins A and E1 did not give precipitin lines in this assay (Fig. 2). We confirmed that the precipitin lines, when formed, were due to specific precipitation of colicin by a combined immunodiffusion-colicin plate test (26; data not shown). Proteins corresponding to the putative colicins E2 through E7 were the major proteins precipitated from solution by the antiserum, but other proteins could also be detected by SDS-polyacrylamide gel electrophoresis (up to 10% of the total nonserum proteins precipitated). Many of these precipitated polypeptides comigrated in the gel with proteins normally present in culture media of induced cells (27). The anti-colicin E3 serum also prevented the bactericidal activity of colicins E2 through E7, and the titer of the neutralizing activity was the same (1/256) for each of these colicins under the conditions used.

TABLE 2. Effects of mitomycin C, catabolite repression, and *recA56* on colicin production[a]

Colicin	Lacuna frequency[b] (%)	Relative lacuna frequency[c]					Colicin yield[d]	
		+ Mitomycin C	+ Glucose	+ Glucose + cyclic AMP	*recA56*	*cya crp*	Control	+ Mitomycin C
A	0.07	229	0.07	0.9	2×10^{-5}	0.002	1	320
E1	0.06	817	0.02	1.4	5×10^{-6}	0.004	2	2,600
E2	0.30	330	0.23	0.81	1×10^{-3}	0.02	200	3.2×10^4
E3	0.02	4,950	0.03	0.91	3×10^{-4}	0.003	80	4,000
E4	0.73	136	0.08	0.72	0.01	0.007	4	400
E5	0.05	1,920	0.02	1.4	2×10^{-5}	0.0006	32	1.6×10^4
E6	0.08	1,125	0.01	1.6	2×10^{-5}	0.003	32	3.2×10^4
E7	0.31	319	0.02	0.77	8×10^{-4}	0.005	800	4,000

[a] Cultures were grown in tryptone broth supplemented, where indicated, with 0.5% glucose and 1 mM cyclic AMP. Mitomycin C (500 ng/ml) was added at an optical density at 550 nm of 0.3.

[b] Lacuna counts were determined after 40 min of continued incubation and are expressed as percent lacuna plus colony counts.

[c] The effects of mitomycin, glucose, cyclic AMP, and *recA56* are expressed relative to figures obtained with untreated wild-type Col^+ cells. The effect of *cya* and *crp* mutations is expressed relative to isogenic cya^+ crp^+ cultures.

[d] Colicin yields were determined after 120 min of incubation (27) and are expressed as arbitrary units derived from the endpoint spot dilution assay. Actual yields of colicin E4 were probably higher than indicated because this colicin is rapidly degraded upon release from Col^+ cells.

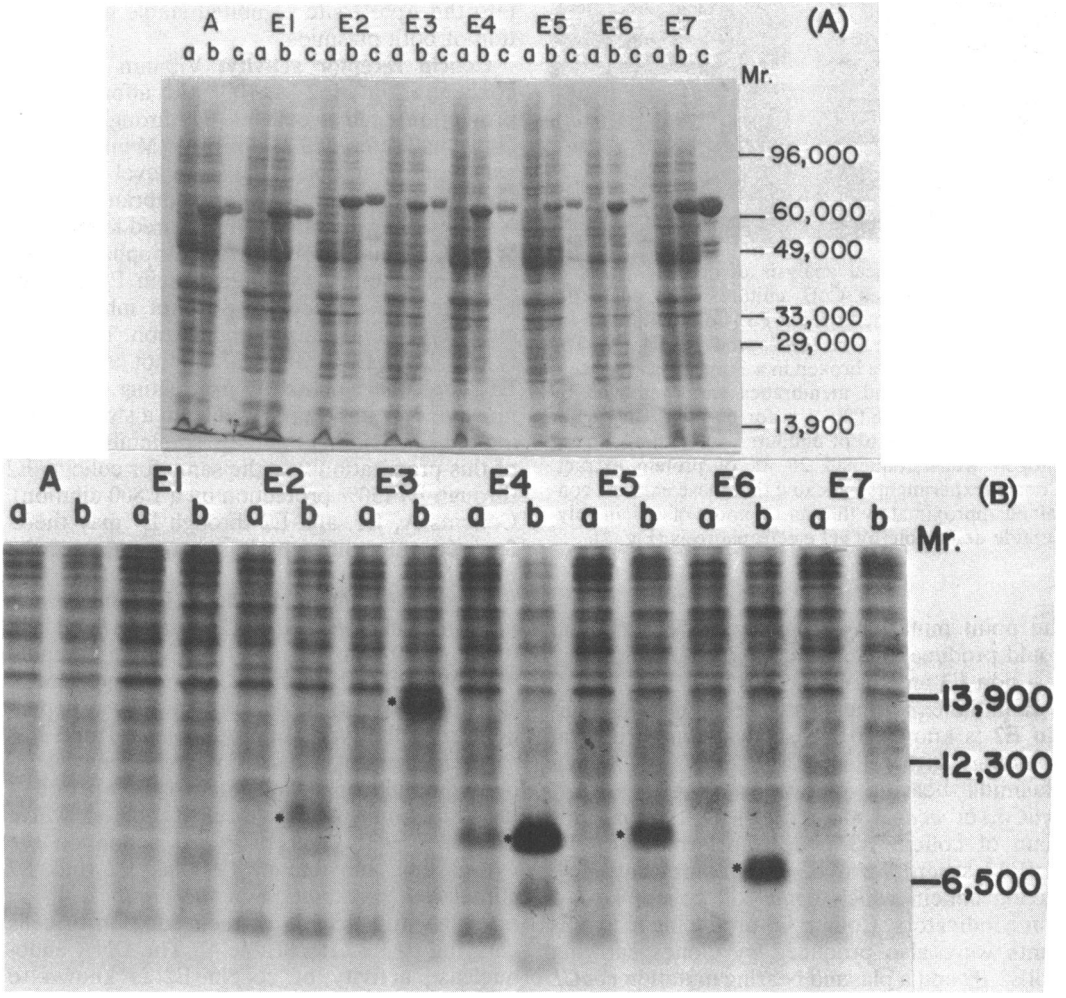

FIG. 1. Identification by SDS-polyacrylamide gel electrophoresis of proteins whose production is stimulated by mitomycin treatment. A, Coomassie blue-stained 10% acrylamide gels displaying proteins present in Col^+ cells grown in tryptone broth (a) or in tryptone broth containing 500 ng of mitomycin per ml for 60 min (Col $E2^+$ through $ColE7^+$) or 120 min ($ColA^+$ and $ColE1^+$; b). Cells were washed once in 20 mM Tris buffer (pH 7.1 with HCl), suspended in sample buffer (29), and heated to 100°C for 5 min. Samples equivalent to 150 μl of culture were loaded. Samples c are partially purified colicins prepared from the same strains. The major bands present in b and c and present only at low levels in a are tentatively identified as the colicins. Differences between electrophoretic mobilities of the putative colicins in whole cell extracts and in the partially purified preparations are due to overloading in slots a and b. B, Autoradiograph of low-molecular-weight proteins synthesized by Col^+ cultures in the presence of ^{14}C-amino acids. Samples prepared as above were resolved by electrophoresis in 14% acrylamide gels containing 6 M urea. a, Cells grown in the absence of mitomycin; b, cells growing in the presence of 500 ng of mitomycin per ml. Only the autoradiograph of the lower part of the gel is shown. Proteins present in larger amounts in mitomycin-grown cultures (*) presumably represent immunity proteins (37). Molecular weight standards (in order of decreasing molecular weight) were phosphorylase a, catalase, fumarase, aldolase, bovine carbonic anhydrase B, lysozyme, cytochrome *c*, and bovine pancreatic trypsin inhibitor.

Undiluted antiserum did not affect the activity of colicins A or E1.

Recombination among the colicin genes. In these experiments we attempted to obtain recombinants between derivatives of ColE3.CA38 encoding inactive colicin polypeptides and wild-type colicin genes on other Col plasmids. It should be noted, however, that the appearance of such apparently hybrid colicins gives no indication as to the amount of each of the parental colicin genes incorporated into the recombinant gene. A double-crossover event in the region of

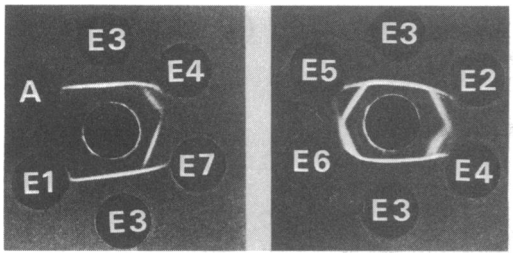

FIG. 2. Serological analysis of crude extracts of mitomycin C-treated Col$^+$ cultures with antiserum raised against purified colicin E3. Col$^+$ cultures were grown as described in the legend to Fig. 1. The harvested cells were broken in a French pressure cell, and cell debris and membranes were removed by centrifugation at 46,000 × *g* for 60 min. The center wells contained 20 μl of undiluted antiserum, and the antigen wells contained 20 μg of protein extract. Control experiments indicated that these extracts contained approximately the same amount of colicin polypeptide detectable by gel electrophoresis (Fig. 1).

the point mutation in the mutated *ceaC* gene could produce an active colicin containing very few non-E3 amino acids.

In initial experiments we used ColE2.P9; colicin E2 is known to have substantial structural homology with colicin E3 (9, 23, 35, 41). When plasmids bearing mutations *ceaC4*, *ceaC5*, *ceaC6*, or *ceaC7* affecting the N-terminal domain of colicin E3 were transferred into the ColE2$^+$ strain, we obtained recombinants producing colicin which could kill colicin E2-immune indicators. Colicin E3-producing recombinants were also produced by clones carrying ColE2.P9 and a plasmid bearing mutation *ceaC1* which may affect colicin penetration (22), but not by clones carrying ColE2.P9 and a plasmid bearing mutation *ceaC2* affecting colicin E3 catalytic activity (22).

These experiments were extended to include crosses of plasmids carrying mutations *ceaC1* or *ceaC3* with other Col plasmids. Plasmids ColE5.099 and ColE6.CT14 recombined with either of these mutant ColE3 plasmids to produce active colicin. Recombinants producing active colicin E3 were also obtained in crosses between ColE4.CT9 and plasmids bearing the *ceaC1* mutation, but not the *ceaC3* mutation. Col plasmids encoding colicins A, E1, or E7 did not recombine with either mutant ColE3 plasmid to produce active colicin E3. The result with ColE7.K317 was surprising since cross-neutralization experiments (above) indicated that colicins E3 and E7 were at least partially homologous. It appeared, however, that these two plasmids could not exist stably in the same clone. Strains carrying ColE7.K317 and a derivative of pBR322 with the colicin E3 gene cloned into the *Eco*RI site exhibited stable cosegregation of both plasmids.

Colicin receptor activity. Vitamin B12 at a concentration of 2×10^{-10} M afforded 50% protection against colicins E1 through E7 (see above and reference 25), but 10^{-7} M vitamin B12 was required to obtain the same level of protection against colicin A. Outer membranes (Triton X-100-insoluble walls [29]) prepared from strain W3110, but not those from bacteriophage BF23-resistant mutants, inhibited colicin E1 through E7 activities. Colicin A was not inhibited by either type of membrane preparation. The activity of colicins E2 through E7, but not colicin A or E1, could be blocked by pretreating cells with inactive colicin E3 prepared from a strain carrying the *ceaC1* mutation (22). The inhibitory titer of this preparation was the same for colicins E2 through E7 (50% protection by a 1/800 dilution). Colicins A, E1, and E2 through E7 may therefore recognize different domains on the receptor protein.

Certain colicins, including A, E2, and E3, are inactive against *E. coli* K-12 mutants which lack outer membrane porins (12). Colicin A could only kill cells which produced the OmpF porin. Sensitivity to colicins E2 through E7 was dependent upon the type of porin present (Table 3).

Mechanisms of action. Colicins A and E1 inhibited uptake of proline within a short period of their addition to sensitive cells (Table 4), which is in line with their proposed action on the cytoplasmic membrane. Colicins E2 and E7 stimulated transcription of the *cia-lacZ* fusion operon within 15 min of their addition to cells carrying pAPBZ153 (Table 4). The DNA endonuclease activity of colicin E2 is known to induce the so-called SOS DNA repair system, with consequent induced transcription of a number of operons of which *cia-lacZ* is one (24). It is therefore likely that colicin E7 resembles colicin E2 with respect to its endonucleolytic activity (30). Colicins A, E1, and E3 through E6 did not stimulate *cia-lacZ* transcription, but inhibited synthesis of preinduced β-galactosidase encoded by the chromosomal *lacZ* gene (Table 4). Colicins E3, E4, E5, and E6 also inhibited the incorporation of ^{14}C-amino acids into cold trichloroacetic acid-precipitable material (Table 4).

DISCUSSION

The eight colicins tested all exhibited reduced activity against BtuB mutants of *E. coli* K-12. We therefore propose that they be referred to as the BtuB group. Colicins A and E1 have very little or no structural homology with colicin E3, as determined by serological studies and by the lack of recombination between mutant colicin E3 genes and wild-type colicin A or E1 genes. Colicins E2 and E4 through E7 appear to be

TABLE 3. Colicin sensitivity of *E. coli* K-12 mutants producing different porins

Strain/porin present[a]	Relative colicin sensitivity[b]							
	A	E1	E2	E3	E4	E5	E6	E7
CS489/OmpC	$<10^{-4}$	1	0.125	0.125	1	0.125	0.062	0.062
CS484/PhoE	$<10^{-4}$	1	0.031	0.015	0.062	1.25×10^{-4}	0.015	$<10^{-4}$
CS483/NmpC	$<10^{-4}$	1	0.062	0.015	0.062	7.8×10^{-3}	0.015	$<10^{-4}$
CS482/none	$<10^{-4}$	1	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-4}$

[a] Protein terminology and strain descriptions are given in reference 29, except that NmpA protein is now called PhoE.

[b] Colicin titers were determined by the endpoint dilution spot assay. Colicin sensitivity is given relative to strain CS490 (OmpFp$^+$). <, Strain was not affected by the highest colicin concentration tested.

more closely related to colicin E3 by these criteria.

The possibility that colicins E3 and E6 may have similar catalytic domains is strengthened by the recent observation that *E. coli* K-12 strains producing cloacin DF13, which also has ribonucleolytic activity (6), are immune to colicin E6 (18a). If colicins E3, E5, and E6 do indeed have homologous catalytic domains, the binding sites for their respective immunity proteins must be different. The immunity proteins for cloacin DF13 and colicin E3 differ only in a few amino acids (39). We were, however, unsuccessful in attempts to obtain mutations in the colicin E3 immunity gene which conferred immunity to other colicins in the BtuB group.

Watson (40) has presented partial sequence analyses of the catalytic C terminal domains of colicins A, E1, Ia, and K, which have similar modes of action (16; F. Pattus, personal communication). It has been suggested that these colicins may have a common evolutionary origin, with mutations and recombination events leading to the appearance of catalytically similar colicins which bind to different cell surface receptors. We might extend this hypothesis to explain the apparent homology of colicins E2 through E7 and possibly also cloacin DF13. Homologous receptor recognition domains (E2 through E7) are apparently combined with at least two, and possibly three, catalytic domains and six immunity domains.

TABLE 4. Effects of colicins on sensitive cells

Colicin[a]	Relative activities[b]			
	Proline uptake[c]	Protein synthesis[d]	β-Galactosidase	
			lacZ[e] + IPTG[f]	*cia-lacZ*[e]
A	0.06	0.05	0.12	0.9
E1	0.07	0.09	0.07	0.9
E2	0.96	0.84	0.74	6.7
E3	1.04	0.02	0.04	0.8
E4	1.08	0.07	0.06	0.9
E5	0.98	0.03	0.11	0.9
E6	1.01	0.09	0.11	0.9
E7	1.04	0.76	0.92	4.6

[a] Partially purified colicins were used at the lowest concentration which gave less than 4% survival (determined by plate counts) after 5 min and which completely inhibited growth (measured turbidometrically) in the period immediately after colicin addition. The use of higher colicin doses did not affect the results obtained. Incubation for 30 to 120 min in the presence of colicins E2 through E7 eventually reduced amino acid uptake.

[b] Rates are relative to untreated control cells.

[c] Initial proline uptake rate in control cells: 80 to 150 pmol/10^8 cells per min.

[d] ^{14}C-amino acid incorporation in untreated cells: 8×10^3 to 1.9×10^4 cpm/10^9 cells per min for 5 min.

[e] β-Galactosidase levels in untreated cultures rose from 2 to 25 to 40 U/ml after 30 min incubation. IPTG, Isopropyl-β-D-thiogalactopyranoside.

[f] β-Galactosidase activity in untreated cultures of pAPBZ153$^+$ rose from 60 to 65 U/ml after 30 min. β-Galactosidase activity in cells treated with 500 ng of mitomycin per ml rose to 426 U/ml after 30 min.

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