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Identification and Characterization of Col Plasmids from Classical Colicin E-Producing Strains†

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A series of transformants have been derived which carry the Col plasmids from 11 E-colicin-producing strains isolated by Fredericq or Hamon. The ColE plasmids identified included four of type E1, five of type E2, and two of a type designated E4 by Horak (Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **233**:58-63, 1975). Strain K317 was shown to carry a ColE7 plasmid and a 2.7-megadalton (Md) $ColE2_{imm}$ plasmid which confers immunity to colicin E2 but not the ability to produce colicin. Other plasmids identified in the colicinogenic isolates were a 3.4-Md ColN plasmid and a 1.3-Md Col plasmid of unknown type. The ColE1 plasmids all continued replicating in cultures treated with chloramphenicol. Strains carrying the ColE4 or ColE3-CA38 plasmid exhibited partial sensitivity to their own colicins and exhibited an unusual clearing-zone morphology when overlaid on stabs of colicin E2- or E7-producing strains. Except for ColE1-K53, ColE1-K47, ColE2-CA42, and ColE7-K317, all of the ColE plasmids were found to be about 4.3 Md in size. ColE2-CA42 and ColE7-K317 are both about 3.9 Md and are the only two plasmids of the E2, E3, E4, and E7 types that did not yield a 0.39-Md deoxyribonucleic acid fragment as an *Eco*RI digestion product. The comparisons of the ColE plasmids suggest several structural and functional relationships among them.

Many isolates of enteric bacteria produce bactericidal proteins known as colicins. The first systematic classification of colicin types was made by Fredericq (10), who has defined one group, the E colicins, as those unable to kill a class of resistant mutants now known to lack the outer membrane protein specified by the *btuB* gene (1, 11). (The exceptions are those otherwise classified as A colicins [8]). The colicin E-producing strains were subdivided on the basis of their immunities, i.e., specific insensitivities to their own or closely related colicins (12). Colicin E1 producers were defined as those incapable of killing K12-30, an *Escherichia coli* K-12 strain made colicinogenic by conjugation with the isolate K30. Colicin E2 producers were defined as those not killing K12-317, derived in the same way by using the isolate K317. Those killing both these strains were assigned to a third group, type E3. The colicin E3 producers, then, were not necessarily related by their immunities, though this designation has since become specifically associated with the immunity displayed by strains carrying the ColE3 plasmid from strain CA38 (21).

Colicin E production and immunity are known to be conferred by plasmids, as these phenotypes can often be transferred from cell to cell with the help of a companion conjugative plasmid (see references 13 and 30 for reviews). However, other than ColE1-K30, ColE2-P9, and ColE3-CA38, few ColE plasmids have been described at the molecular level. The purpose of this study was to derive and characterize a series of transformants carrying ColE plasmids from a variety of *E. coli* isolates producing E colicins. We analyzed the plasmids in a collection of strains isolated by Fredericq or Hamon, including three strains producing colicin E1 and four producing colicin E2. One, CA42, makes a colicin E2 which can be distinguished immunologically from colicin E2-P9 (24). Strains K321, K365, and 284 were also chosen for study because they produce incompletely defined E colicin types. Of these, Reeves has suggested that K321 may produce colicin E2 plus another E colicin (29), and 284 is known to additionally produce colicin N (8, 15, 23). Another strain analyzed was K317, described above, recently shown to produce a new colicin type, E7, and inferred to carry a plasmid

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imparting immunity to colicin E2 but not its production (26). Since these 11 strains and their colicins have been the subjects of a number of studies (8, 9, 15, 20, 23, 24, 26, 28, 29, 31), identification of their Col plasmids allows them to be associated with the colicinogenic properties which have been described.

MATERIALS AND METHODS

Bacterial strains and plasmids. Table 1 shows the bacterial strains and plasmids used as defined colicin producers and indicators. The colicinogenic isolates, referred to as "parent" strains, are listed in Table 2, together with the former identification of their colicins. They are *E. coli* strains from the collection of P. Reeves, though K53, CA42, and 284 were obtained via E. Lederberg, the Plasmid Reference Center, Stanford, Calif. Strain 285 was also obtained from P. Reeves. V517 was obtained from F. L. Macrina (25). Plasmid pPM103 is a pSC101 derivative which is temperature sensitive for replication and imparts tetracycline resistance (27). This plasmid in strain C600 was obtained from E. Lederberg.

Bacterial growth conditions and media. L-broth and L-broth bottom and top agar for plates were prepared as described previously (34) and used for all colicin and immunity testing. Dilute L-broth agar contains 100 ml of L-broth, 900 ml of water, 8 g of NaCl, and 17 g of agar per liter. Plates containing this medium give overnight growth of 1- to 2-mm-diameter colonies and were used for transformant selections and replica plating. When required, tetracycline was added to 10 μg/ml. Unless otherwise indicated, all bacterial growth was at 37°C.

M9 medium contains 7 g of Na_2HPO_4, 3 g of KH_2PO_4, 1 g of NH_4Cl, and 1 g of NaCl per liter, with 10 ml each of sterile 0.1 M $MgSO_4$ and 0.01 M $CaCl_2$ added after autoclaving. This medium, supplemented with 0.2% glucose, 20 μg of thiamine per ml, and 0.2% Casamino Acids (Difco), was used to grow cultures for chloramphenicol amplification of their plasmids (6). For this technique, cells were grown exponentially with shaking. Chloramphenicol was added to 170 μg/ml when the culture reached an optical density of 1.0 at 450 nm, and incubation was continued overnight.

Colicin production and immunity testing. To test a strain's colicin an L-broth plate was streaked or stabbed with cells and incubated overnight. A dry filter-paper circle was placed on the surface of the plate and then removed together with the adhered cells. (This allowed subsequent detection of colicin in the agar beneath them.) The plate was exposed to chloroform vapor for 5 to 10 min, left open for about 2 min, and then overlaid with 3 to 5 ml of melted top agar into which a fresh overnight culture of an indicator strain had been diluted 100-fold. The plate was reincubated for 4 h and then scored for the presence of a clear zone above the area of initial application of cells. A strain's immunity was tested in the same way, except that it was used as the indicator over streaks of strains producing known colicin types. In some tests, partially purified colicin was streaked on the plate, cross-streaked with the strain to be tested, and incubated overnight. Strains sensitive to the colicin did not grow over the area to which it was applied.

Colicins E1-K30, E2-P9, and E3-CA38 were purified as described previously (17, 18, 34). One unit of colicin is the highest dilution producing a clearing when spotted on a top agar overlay seeded with strain WA802.

TABLE 1. *Bacterial strains and plasmids*[a]

Strain	Description	Source	Reference
WA802	Colicin-sensitive indicator *metB1 hsdR2 galK2 galT2 lac-3 lacY1 supE44* λ^-	J. D. Frieson, York Univ., Toronto, Ont.	35
WA802(ColE1-K30)	Colicin E1 producer and immune indicator	By transformation with plasmid from W3110(ColE1-K30)	This study; 16
WA802(ColE2-P9)	Colicin E2 producer and immune indicator	Our collection	34
WA802(ColE3-CA38)	Colicin E3 producer and immune indicator	Our collection	34
WA802(ColE4-CT9)	Colicin E4 producer and immune indicator	By transformation with plasmid from BZB1011(ColE4-CT9)	This study; 19
WA802(pBR322)	Colicin-sensitive indicator which is resistant to tetracycline	By transformation with plasmid from RR1(pBR322)	This study; 4
BZB1011(ColA-CA31)	Colicin A producer and immune indicator	E. Lederberg, Plasmid Reference Center	
BZB1011(ColE4-CT9)	Colicin E4 producer and immune indicator	E. Lederberg, Plasmid Reference Center	
P525	Indicator resistant to E and A colicins; a *btuB* derivative of AB1133	P. Reeves	8

[a] The BZB1011 strains bearing plasmids were derived by A. Pugsley, Biozentrum, University of Basel, Switzerland. BZB1011 is a *gyrA* derivative of W3110. The genotypes of the strains referred to may be found in the references given. The WA802 derivatives carry only the plasmid species indicated.

TABLE 2. *Characteristics of the parent strains and colicinogenic transformant classes*

Parents			Transformants													
					Colicinogenic properties[f]											
					Killing of:						Sensitivity to:					
Strain[a]	Colicin[b] types known	Plasmid mass[c] (Md)	Type[d]	Selection[e]	WA 802	E1	E2	E3	E4	E^R	E1	E2	E3	E4	Parent	Plasmid mass[g] (Md)
K53	E1	1.3, 3.3, >20	E1	Colicin E1	+	−	+	+	+	−	−	+	+	+	+	3.3
			E1 + ?	Colicin E1	+	+	+	+	+	+	−	+	+	+	−	1.3, 3.3
K47	E1	6.5, >20	E1	Colicin E1	+	−	+	+	+	−	−	+	+	+	−	6.5
N104	E1	4.3, >20	E1	Cotransformation	+	−	+	+	+	−	−	+	+	+	−	4.3
GEI288	E2	0.95, 1.8, 4.3, >20	E2	Colicin E2	+	+	−	+	+	−	+	−	+	+	−	4.3
GEI554	E2	2.7, 4.3, >20	E2	Cotransformation	+	+	−	+	+	−	+	−	+	+	−	4.3
GEI602	E2	1.9, 3.7, 4.3, >20	E2	Colicin E2	+	+	−	+	+	−	+	−	+	+	−	4.3
CA42	E2	3.9	E2	Colicin E2	+	+	−	+	+	−	+	−	+	+	−	3.9
K321	E2	0.95, 1.8, 2.7, 3.4, 4.3 (×2), >20	E1	Colicin E1	+	−	+	+	+	−	−	+	+	+	+	4.3
			E2	Colicin E2	+	+	−	+	+	−	+	−	+	+	+	4.3
			E1 + E2	Colicin E1 or E2	+	+	+	+	+	−	−	−	+	+	−	4.3 (×2)
K317	E2	1.3, 1.8, 2.7, 3.9, >20	E7	Cotransformation	+	+	+	+	+	−	+	+	+	+	−	3.9
			$E2_{imm}$	Colicin E2	−	−	−	−	−	−	+	−	+	+	+	2.7
			E7 + $E2_{imm}$	Cotransformation or colicin E2	+	+	+	+	+	−	+	−	+	+	−	2.7, 3.9
K365	E3	1.0, 4.3, >20	E4	Colicin from K365	+	+	+	+	−	−	+	+	+	−	−	4.3
284	E3 + N	2.0, 3..4, 4.3, >20	E4	Colicin from K365	+	+	+	+	−	−	+	+	+	−	+	4.3
			N	Cotransformation	+	−	+	+	+	+	+	+	+	+	+	3.4
			E4 + N	Colicin from K365 or cotransformation	+	+	+	+	+	+	+	+	+	−	−	3.4, 4.3

[a] All strains are *E. coli* isolated by Fredericq except 284, which was isolated by Hamon.

[b] Former colicin classifications as described by Reeves (8, 29). E3 is used here in its original sense, that is, as a type different from E1 or E2.

[c] Plasmids were detected in each strain by the procedure of Birnboim and Doly (3). Error is ±5%. In some cases, such as the two 4.3-Md plasmids in K321, data from the transformation experiments were used to augment this list. No attempt was made to size the plasmids over 20 Md.

[d] Colicinogenic transformant types were inferred from the colicinogenic properties as given in the table or as described in the text. Sixteen to 64 transformants were tested from each experiment, though as few as 3 were of the doubly colicinogenic classes when two Col plasmids were detected.

[e] Selections were performed as described in the text. Colicins E1, E2, and E3 refer to selection with colicins E1-K30, E2-P9, and E3-CA38, respectively.

[f] "Killing of" refers to the ability of the colicin from the transformants to kill the strains indicated. "Sensitivity to" refers to the ability of the colicins from the strains indicated to kill the transformants. +, Killing; −, no killing. WA802, E1, E2, E3, E^R, and Parent are the indicator strains WA802, WA802(ColE1-K30), WA802(ColE2-P9), WA802(ColE3-CA38), P525 and the parent listed in the first column, respectively. E4 refers to later characterizations with WA802(ColE4-CT9).

[g] Plasmids of the molecular weights givens were the only species common to all transformants in each class. In each case 3 to 30 transformants from each class were tested for their plasmid contents to identify strains carrying only the plasmids indicated. Those strains carrying single plasmid species were verified as such by purification of their plasmids and restriction endonuclease digestions. Molecular weight error is ±5%.

Colicin produced by strain K365 was purified by the procedure used for colicin E3-CA38, except that the portion of the cell wash precipitating between 10% and 60% $(NH_4)_2SO_4$ saturation was used.

Plasmid screening techniques. Cultures were screened for their plasmid content by the alkaline extraction procedure of Birnboim and Doly (3), using 1.0% agarose gels for the electrophoretic separation. Strain V517 was used as a source of covalently closed circular plasmids of known molecular masses to calibrate the gels in the range 1.4 to 4.8 megadaltons (Md) (25). This range was extended to span 0.95 to 6.5 Md, using several plasmids carried by the parent strains as markers. These were sized in their linear forms after purification and restriction endonuclease digestions.

Two methods were used to test for a ColE1-K30-like ability to continue replicating in chloramphenicol-treated cells. One was to compare the yields of plasmid DNA obtained from treated cells with those obtained from treated cells containing ColE1-K30. Those called amplifiable gave yields of plasmid DNA of over 1 mg/liter of culture. Non-amplifiable plasmids were obtained at less than 5% of this yield. The second method was to compare the amounts of plasmid DNA in 0.5-ml portions of cultures taken before addition of chloramphenicol with those taken 16 h after the addition, using the plasmid screening technique of Birnboim and Doly (3). The plasmid bands from this amount of untreated cells were faintly visible in the gel. Amplifiable plasmids were visible as heavy overloaded bands after chloramphenicol treatment.

Restriction endonuclease digestions and agarose gel electrophoretic techniques were as described previously (34).

Purification of plasmids. Plasmids not amplifiable by chloramphenicol were purified as described

previously (34), except that stationary-phase cells were used. Amplifiable plasmids were purified from 200 ml of chloramphenicol-treated culture by using the procedure of Guerry et al. (14). The plasmids from the parent strains were purified by the non-amplifiable plasmid technique, with the exception of those of strains N104 and GEI554, for which the procedure described by Birnboim and Doly was used (3).

Bacterial transformation. Transformations were done as described by Dagert and Ehrlich (7), except that the cells were kept on ice for only 30 min after the $CaCl_2$ treatment before they were used. The transformed cells were grown for 1 h to allow phenotype expression before plating. Colicinogenic transformants were selected by spreading dilutions of the culture on dilute L-broth plates freshly spread with 10^3 to 10^4 U of colicin. For indirect selection by cotransformation, 0.1 μg of pPM103 DNA and 0.5 μg of the unselected plasmids were added to the competent cells. After 1 h of incubation at 30°C for phenotype expression, the cells were plated on dilute L-broth plates containing tetracycline and grown overnight at 30°C. Replicas were made onto the same medium, and the master plates were chloroformed and overlaid with top agar seeded with WA802(pBR322). Colonies which produced clearings in the overlays were picked from the replica plate for further testing. The pPM103 plasmid was cured by growing the strains on L-broth plates at 42°C.

RESULTS

Activity of the ColE isolates on indicator strains. The colicinogenic isolates listed in Table 2 were tested as colicin producers against strain WA802, an indicator sensitive to all colicins; against strain WA802 carrying ColE1-K30, ColE2-P9 or ColE3-CA38, indicators which are specifically immune to colicins of types E1, E2, and E3, respectively; and against strain P525, a *btuB* mutant which by its resistance defines colicins of type E and which shows reduced sensitivity to colicin A. Strains K47 and N104 showed killing activity consistent with their classifications as colicin E1 producers. Strains CA42, GEI288, GEI554, and GEI602 killed the indicators in the pattern expected of colicin E2 producers, though GEI554 produced only small clearings, especially when WA802(ColE1-K30) was the indicator. The other five parent strains were able to kill all three colicin-immune indicators. Of these, strains K321, K317, and K365 did not produce colicin active against the *btuB* strain and were inferred to carry either multiple ColE plasmids or a ColE plasmid different from the types E1, E2, and E3-CA38. Strains 284 and K53 were found to produce colicin that killed the *btuB* strain, indicating that they carry Col plasmids of other than the E type, as expected for strain 284, which is known to produce colicins E and N (8, 15, 23), but not for strain K53. The latter behaved as though it produced colicin E1 and a less potent colicin that could be overgrown by the *btuB* strain or the ColE1-carrying indicator strain on prolonged incubation.

The parent strains were also tested for immunity to the colicins produced by the ColE1-K30, ColE2-P9, and ColE3-CA38 strains. Only GEI288, GEI554, and CA42 gave responses indicative of the presence of a plasmid conferring a specific immunity to one of the three colicin types, in these cases colicin E2. K317 was partially sensitive to the colicins produced by all three ColE strains, a result which will be referred to later. The other strains were insensitive or only slightly sensitive to the colicins produced by the tester strains, probably indicating that they had acquired chromosomal mutations giving resistance to them. (Phage BF23 sensitivity was not tested.)

Plasmids of the parent strains. To determine the plasmid content of the 11 parent strains, extracts containing their covalently closed circular DNA species were prepared as described in Materials and Methods and analyzed by agarose gel electrophoresis. The sizes of the plasmids, determined by comparison of their electrophoretic mobilities with those of known molecular weights, are given in Table 2. A 3.9-Md plasmid was the only species detected in strain CA42, in agreement with the results of Hughes et al. (20). Each of the other strains was found to carry from one to six small plasmids ranging in size from 0.95 to 6.5 Md, together with a larger plasmid of undetermined molecular weight.

Hamon's strain 284 was found to have four plasmids (Table 2). A strain called 285, a colicin E and N producer also isolated by Hamon (8, 15, 23), not included in Table 1 or 2, was also tested and found to be identical to 284 in plasmid content and colicinogenic properties.

Transformation of Col plasmids. To identify the Col plasmids in the parent strains, plasmid DNA from each was used to transform WA802. Colicinogenic transformants were obtained either by selecting for their immunity to colicin or by cotransformation with the plasmid pPM103. These were then tested for colicin and immunity characteristics and plasmid content (Table 2). The molecular weights of the Col plasmids identified are given in Table 2; in each case, transformants were found carrying only the one or two plasmids species listed. The colicinogenic transformants inactive against the *btuB* strain were designated as carriers of ColE rather than ColA plasmids since it was demonstrated that they are sensitive to the colicin produced by BZB1011(ColA-CA31).

Singly colicinogenic colicin E1 or E2 producers. When prepared with plasmid DNA

from strain K47 or N104, all of the colicinogenic transformants obtained were inactive against WA802(ColE1-K30). Similarly, only colicin E2-producing transformants were obtained when we used plasmid DNA from strain GEI288, GEI554, GEI602, or CA42. Strains carrying single plasmid species were immune to the colicin of their parent strain, indicating that the six parent strains are singly E1 or E2 colicinogenic, a result consistent with their killing properties. Unlike the parent strain GEI554, the transformants carrying ColE2-GEI554 produced large clearings in sensitive strains, including the indicator strain carrying ColE1-K30, as did the other ColE2 transformants. It appears that the reduced activity of this plasmid in the parent strain is due to some host character.

K53 Col plasmids. By using strain K53 plasmid DNA, the transformants selected on plates containing colicin E1 were of two classes: one produced only an E1 colicin, and the other produced both an E1 colicin and a colicin of another type. The ColE1 plasmid was identified as a 3.3-Md species present in both transformant classes. The non-E colicinogeny was correlated with the presence of a 1.3-Md plasmid. No attempt was made to obtain transformants carrying this small Col plasmid alone, nor have we identified its colicin type.

K321 ColE plasmids. By using strain K321 plasmid DNA, transformants were obtained on plates spread with either colicin E1 or colicin E2. Testing revealed classes immune to colicin E1 or to colicin E2 and a class immune to both of these colicin types, obtained from both types of selective plates. Only the doubly colicinogenic transformants were immune to the colicin produced by strain K321. Thus K321 carries both ColE1 and ColE2 plasmids, which explains its ability to kill the three ColE indicator strains. All three transformant classes contained 4.3-Md plasmid species, inferred to be ColE1 and ColE2 plasmids of about the same molecular weight. The nonidentity of these two plasmid species was verified by restriction endonuclease analysis (data not shown).

K365 and 284 ColE plasmids. No transformants were obtained when competent cells treated with plasmid DNA from strain K365 were spread on plates containing colicin E1, E2, or E3. As this indicated that K365 carried a type of ColE plasmid distinct from these three, colicin was prepared directly from this strain and used for the selection. Transformants were readily obtained and found to have only the 4.3-Md plasmid from the parent in common. Further testing of a transformant carrying this ColE plasmid verified that it conferred immunity to the colicin of the parent, K365, but not to colicin E1-K30, E2-P9, or E3-CA38.

There have been several reports designating an E colicin immunity type E4 (5, 19; cited in reference 30). Of these, only Horak's colicin E4 + Ia strain (colicin type 9 strain) (19) has been confirmed as producing an E colicin different from types E1, E2, and E3-CA38 (B. Males and B. A. D. Stocker, Stanford University, personal communication). By using BZB1011(ColE4-CT9), a strain derived by A. Pugsley, which carries the ColE plasmid from Horak's strain, and WA802(ColE4-CT9), a transformant derived by us (Table 1), the strain WA802 transformants carrying the ColE plasmid from strain K365 were shown to produce and to be immune to colicin E4. Thus the plasmid was designated ColE4-K365. ColE4-CT9 was also found to have a molecular mass of 4.3 Md.

Because strain 284 has been described as a producer of colicins E and N, cotransformation was used in an attempt to obtain transformants carrying each of the Col plasmids presumed to be present in the strain. However, all of the colicinogenic transformants picked by this technique were found to be active against the *btuB* strain, demonstrating that none carried a ColE plasmid alone. The killing of the *btuB* strain was inferred to be due to a 3.4-Md plasmid, presumably ColN-284, present in all the transformants. A transformant carrying this plasmid alone, WA802(ColN-284), produced small clearing zones which were not always observable, though it was usually found to kill WA802, the *btuB* strain P525, and all of the WA802 derivatives carrying ColE plasmids of other than the E1 type. Killing of WA802(ColE1-K30) and the other E1 type strains was never observed, but WA802(ColN-284) was killed by the E1 colicins.

Some of the colicinogenic transformants obtained by cotransformation were immune to the colicin of the parent strain, 284, showing that they carried both of its Col plasmids. On testing they proved to be immune to colicin E4, thus identifying the ColE plasmid as an E4 type and allowing the direct selection of transformants carrying this plasmid by plating on colicin E4-K365. ColE4-284 was identified as a 4.3-Md plasmid. Screening of the plasmids of some of these transformants is shown in Fig. 1.

When colicin E4-K365 was used to select the ColE4-284 transformants it was noted that the colonies of the singly colicinogenic ColE transformants were smaller than those of transformants carrying both the ColE and ColN plasmids. This difference may explain our failure to pick singly colicinogenic ColE transformants by using the cotransformation technique, as the 30°C growth used in this procedure would further reduce their detectability.

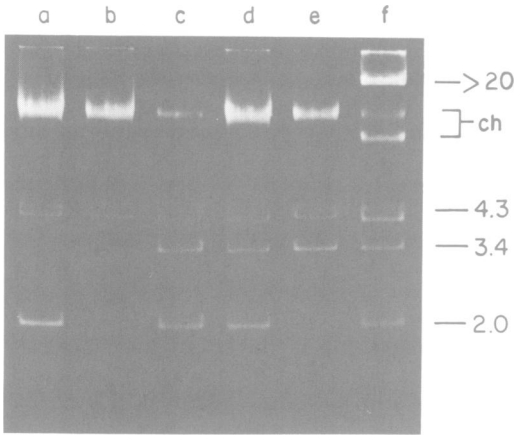

FIG. 1. *Plasmid contents of transformants carrying the ColE plasmid from strain 284. Competent cells were treated with plasmid DNA from strain 284, and transformants were selected on plates spread with colicin purified from strain K365. Shown are plasmid extracts from five transformants (a to e) and strain 284 (f), separated electrophoretically on a 1.0% agarose gel. The plasmids separated in lanes a and b are from transformants producing only an E colicin. Those in lanes c to e are from transformants producing both E and N colicins. The sizes of the plasmids are given in Md. The 4.3-Md ColE plasmid has separated into two bands on this gel. The ColN plasmid is the 3.4-Md band. ch indicates chromosomal DNA bands.*

The ColE7 and ColE2$_{imm}$ plasmids of K317. The ability of the colicin E2-immune transconjugant K12-317 to kill ColE2-carrying strains was initially described by Lewis and Stocker (24). Recently, Males and Stocker have derived transconjugants from strain K317 displaying this killing activity but lacking the colicin E2 immunity phenotype, thus demonstrating that this strain carries a new E-type plasmid, ColE7 (26). Transformants derived here using strain K317 plasmid DNA demonstrated colicin and immunity activities distinct from those of the strains carrying ColE1, E2, E3, and E4. The ColE7-K317 plasmid was identified as the 3.9-Md plasmid in strain K317.

Since a colicin E2 immunity phenotype was displayed by strain K12-317 and some of its transconjugants, Males and Stocker concluded that another plasmid, ColE2$_{imm}$-K317, is also present in strain K317. This has been confirmed by the identification of transformants carrying only the 2.7-Md plasmid from K317 which are specifically immune to colicin E2, yet do not produce colicin (Table 2). In accord with the previously noted observation that strain K317 was partially sensitive to the colicin E2-producing strain, the immunity conferred by ColE2$_{imm}$-K317 was found to be incomplete. Strains WA802(ColE2$_{imm}$-K317) and WA802(ColE2$_{imm}$-K317)(ColE7-K317) appeared partially sensitive when used as indicators in overlays on colicin E2-P9 producers, showing turbid clearings which were smaller than those observed when WA802 or WA802(ColE7-K317) was used in the overlays. The immunity phenotype was demonstrated more conclusively by cross-streaking strains on plates streaked with cell-free colicin E2 preparations. Strains K317, WA802(ColE2$_{imm}$-K317), WA802(ColE2$_{imm}$-K317)(ColE7-K317), and WA802(ColE2-P9) grew over the colicin streak; WA802 and WA802(ColE7-K317) did not. The difference in results from the two testing methods probably reflects the higher colicin concentrations produced around stabs of the ColE2-P9 strain.

Properties of the ColE transformants. Including the indicator strains carrying ColE1-K30, ColE2-P9, and ColE3-CA38 and the strain WA802(ColE4-CT9), 16 different ColE plasmids are represented in the series of WA802 transformants. Each was tested against all the others. Each strain was inactive against all others carrying plasmids of the same type and active against all others carrying only plasmids of different type.

Strains carrying ColE3-CA38 or ColE4 plasmid were found to be somewhat sensitive to their own colicins since they produced turbid clearings when tested against themselves. Stocks of these strains often accumulated colicin-resistant mutants which sometimes predominated in heavily grown cultures or stabs which were transferred serially. Furthermore, single-colony reisolates tended to produce only small clearings on sensitive indicator strains.

The strains carrying the ColE3-CA38 or ColE4 plasmid were found to exhibit a distinctive clearing-zone morphology when used as indicator strains over stabs of any of the ColE2 strains or the ColE7-K317 strain. As shown in Fig. 2, their growth was inhibited around the colicin zone in such a way as to produce two concentric circular clearings. Other combinations of these strain types gave circular clearings with a sharp boundary, such as is shown in Fig. 2E.

Properties of the ColE plasmids. The ColE plasmids carried by the series of transformants have been tested for the ability to continue replicating in chloramphenicol-treated cells, as ColE1-K30 does (6). All of the ColE1 plasmids underwent substantial replication in cells treated with chloramphenicol, whereas the ColE plasmids of types E2, E3, E4, and E7 did not.

ColE2-P9 and ColE3-CA38 are 80 to 90% homologous, and both are cleaved by restriction

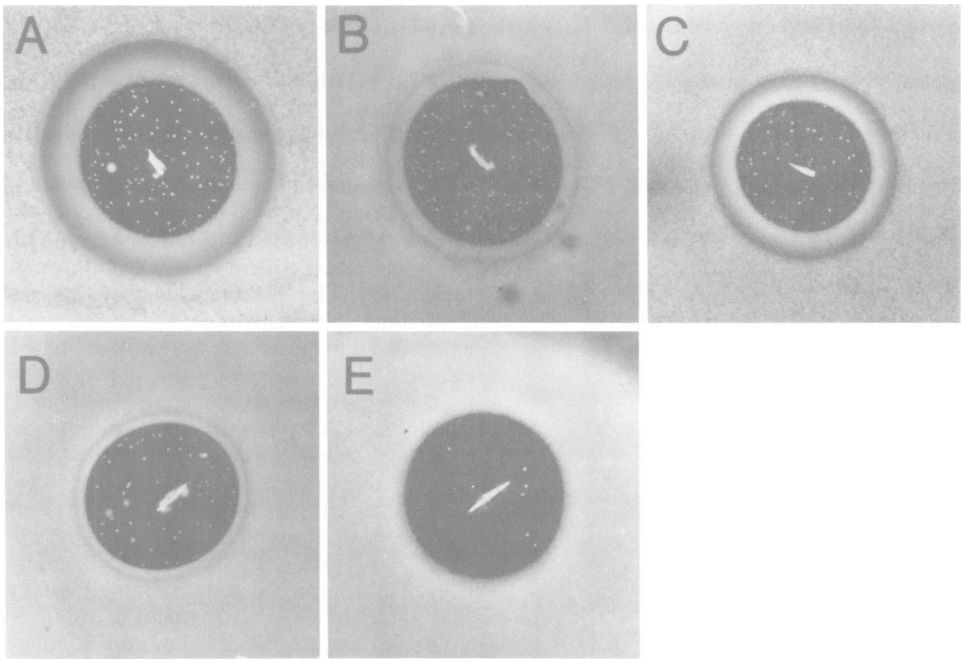

FIG. 2. *Clearing zones characteristic of ColE3- and ColE4-carrying strains overlaid on stabs of colicin E2 or E7 producers. (A) WA802(ColE2-P9) stabbed, WA802(ColE3-CA38) in overlay; (B) WA802(ColE2-P9) stabbed, WA802(ColE4-K365) in overlay; (C) WA802(ColE7-K317) stabbed, WA802(ColE3-CA38) in overlay; (D) WA802(ColE7-K317) stabbed, WA802(ColE4-K365) in overlay; (E) WA802(ColE2-P9) stabbed, WA802(ColE7-K317) in overlay. The plates were illuminated obliquely from below so that the regions of no growth in the overlay appear as the black circles. Resistant mutants are present. The dark outer rings in A–D are regions which are only slightly turbid with cells.*

endonuclease *Eco*RI to produce a 0.39-Md DNA fragment (22, 34). Four of the ColE2 plasmids and the three ColE4 plasmids also yielded *Eco*RI fragments of this size. ColE1-K321, ColE1-K53, ColE1-N104, and ColE2-CA42 were not cleaved by *Eco*RI. ColE1-K30, ColE1-K47, and ColE7-K317 were cleaved once. Table 3 summarizes the data involving the molecular properties of the ColE plasmids.

DISCUSSION

The colicinogenic *E. coli* strains were found to carry an assortment of plasmids other than those identified as determinants of E colicin production. Some of the large plasmids in these strains may be conjugative types, since colicinogenic isolates are frequently competent for plasmid transfer (13). However, among the strains studied, only K317 has been reported to carry such a plasmid, this being pRES-K317, which was described by Males and Stocker as a conjugative plasmid able to restrict phages BF23 and T5 (26). Of the smaller plasmids, a 3.4-Md species was identified as a ColN plasmid, the designation N being that given by Hamon for the heat-stable colicins produced by strains 284 and 285 and three other multiply colicinogenic strains (15, 23). Also identified was a 1.3-Md Col plasmid in K53, though the expression of colicin by this plasmid was not demonstrated in a strain lacking ColE1-K53.

The transformant carrying ColE2$_{imm}$-K317 exhibited immunity to colicin E2, yet did not produce a colicin. Though this immunity was not as complete as that of the ColE2 transformants, its specificity and the small size of ColE2$_{imm}$-K317 (2.7 Md) suggest that it may have been derived from a ColE2 plasmid by a deletion which destroyed its colicin-producing ability.

The ColE1 plasmids all replicated in chloramphenicol-treated cells, as does pMB1, a 6.8-Md ColE1 plasmid carrying the *Eco*RI restriction and modification genes (2). Colicins E1-N104 and E1-K30 are similar in their pattern of inhibition of macromolecular synthesis of sensitive cells (29). Though uniform in these aspects, the E1-type Col plasmids vary more in size (3.3 to 6.5 Md) than do the other ColE types described here (Table 3).

The six ColE2 plasmids studied were homo-

TABLE 3. *Properties of the ColE plasmids*

Plasmid	Mass[a] (Md)	Chloramphenicol amplifiable[b]	0.39-Md *Eco*RI fragment[c]
ColE1-K30	4.2	+	−
ColE1-K53	3.3	+	−
ColE1-K47	6.5	+	−
ColE1-K321	4.3	+	−
ColE1-N104	4.3	+	−
ColE2-P9	4.4	−	+
ColE2-GEI288	4.3	−	+
ColE2-GEI554	4.3	−	+
ColE2-GEI602	4.3	−	+
ColE2-K321	4.3	−	+
ColE2-CA42	3.9	−	−
ColE3-CA38	4.6	−	+
ColE4-K365	4.3	−	+
ColE4-284	4.3	−	+
ColE4-CT9	4.3	−	+
ColE7-K317	3.9	−	−
$ColE2_{imm}$-K317	2.7	−	−

[a] The sizes of ColE1-K30, ColE2-P9, and ColE3-CA38 are taken from the literature (34). Sizes of the other plasmids are from Table 1. The 0.1-Md size differences of ColE1-K30 and ColE2-P9 from the 4.3-Md sizes of others is less than experimental error.

[b] +, Yields of plasmid DNA from chloramphenicol-treated cells comparable to those of ColE1-K30; −, yields less than 5% of ColE1-K30.

[c] +, 0.39-Md *Eco*RI fragment produced by digestion with this enzyme; −, not produced.

geneous by several criteria. Reeves studied the mode of action of the colicins from all six parent strains and found DNA synthesis to be their primary target in vivo (29). These results suggest that they are similar to colicin E2-P9, which degrades DNA in vivo and in vitro (32). The transformants carrying ColE7-K317 or the ColE2 plasmid released colicin which produced the unusual zones of clearing of ColE3 and ColE4-carrying strains shown in Fig. 2. This phenomenon suggests similarity of the E2 colicins and colicin E7-K317. The relatedness of the E2 and E7 colicins is also suggested by their comparable in vivo effects on DNA metabolism (9, 29) and by the partial immunological relatedness of colicins E2-P9 and E7-K317 (24). In the same studies colicin E2-P9 antisera did not inactivate colicin E2-CA42. Thus colicins E2-CA42, E2-P9, and E7-K317 exhibit diverse relationships. ColE2-CA42 is the only plasmid distinguishable from the other ColE2 species by its molecular mass, 3.9 Md, which is the same as that found for ColE7-K317. These two plasmids were also the only two of the E2, E3, E4, and E7 types which did not yield a 0.39-Md fragment after *Eco*RI digestion. Our work in progress has shown that the similarity of these two plasmids also extends to the sizes of some of their restriction fragments.

The strains K365 and 284 were found to carry ColE plasmids of colicin immunity types the same as those of the ColE plasmid from Horak's *Shigella sonnei* colicin type 9 strain, producing colicin E4. The plasmids ColE4-CT9, ColE4-K365, and ColE4-284 were identical in the properties studied here. All were about 4.3 Md in size, and strains carrying these plasmids showed partial sensitivity to their own colicins. Like colicin E3-CA38, the E4 colicins may act by blocking some step in protein synthesis, since colicin from strain K365 has been shown to inhibit this process in sensitive cells (29). The ColE4 plasmids are similar in size to five of the ColE2 plasmids, and like these plasmids and ColE3-CA38 they are cleaved by *Eco*RI such that a 0.39-Md *Eco*RI fragment is produced.

Overall the ColE2, E3, E4, and E7 plasmids appear to be more closely related to one another than to the ColE1 plasmids, though further studies are required to determine the degree to which this is true. The strains derived here should prove useful for this purpose.

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