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Escherichia coli K12 Strains for Use in the Identification and Characterization of Colicins

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A collection of strains derived from *Escherichia coli* K12 W3110 and harbouring various colicin or microcin plasmids (18 and 2 representatives, respectively), or carrying well-characterized mutations conferring reduced colicin/microcin sensitivity is described. The strains can be used in typing schemes based on the identification of colicins, in the detection of new types of colicins/microcins, and in the further characterization of previously identified colicins/microcins and their plasmids.

INTRODUCTION

Bacterial antagonists or toxins which act on strains closely related to the producing organism continue to provoke a great deal of interest. The best studied examples of this type of toxin are the colicins and their close relatives, the microcins (Pugsley, 1984). We are primarily interested in the molecular aspects of colicin production and action, and as part of this work have constructed a set of strains, each carrying a plasmid coding for the production of a single type of colicin or microcin. Various groups working on other aspects of colicinogeny and related phenomena have expressed interest in these strains, and it appears that they may be particularly useful for the identification of colicins produced by new isolates (see Achtman *et al.*, 1983; Watson *et al.*, 1981; Cooper & James, 1984 for examples of cases where strains in our collection have been used for this purpose). In addition, a standardized collection of colicin producer and indicator strains is desirable if the mis-identification of commonly used colicins is to be avoided (Luria, 1982). The colicin-producing strains in the collection have been catalogued, and the catalogue enlarged by the addition of selected colicin-insensitive mutants of *E. coli* K12. The complete set, or parts thereof, are available from Dr E. Lederberg, The Plasmid Reference Center, Department of Medical Microbiology, Stanford University Medical School, Stanford, CA 94305, USA, or directly from the author, who will also make available the supplementary material described here. The present paper describes strains which are in the collection and the various ways in which they can be used.

METHODS

Strains. E. coli K12 strain W3110 and its nalidixate-resistant (*gyrA*) derivative BZB1011 were used as sensitive indicator strains, as hosts for Col and Mcc plasmids, and as starting strains for the selection of colicin-insensitive mutants. *E. coli* K12 strain BZB1019 (*met hsdR gal rpsL*) was used as the Col plasmid recipient in the initial stages of the construction of the Col$^+$ strains. Most Col$^+$/Mcc$^+$ strains are listed in the text and Table 1; additional strains were W3110 *btuB* (pColE1-K30) (from K. Hardy, Biogen SA, Geneva, Switzerland); *E. coli* O6 (pColA-23) (from E. Lederberg); *E. coli* CA42 (pColE2-CA42) (P. Fredericq, University of Liege, Belgium, via P. Reeves, University of Adelaide, Australia); *E. coli* K12 M32 T19 (*tonA* λ^+ pColM-K260) (P. Fredericq via B. Stocker, Stanford University, Calif., USA); *E. coli* K12 pColX-CA23 and *E. coli* K12 pColX-W7 (both from P. Fredericq); and *Shigella dispar* P14 (pColS4-P14) (P. Fredericq via P. Reeves). *E. coli* K12 GW1000 (*lacΔ169 thr leu his arg ilv mtl galK rpsL recA441 sfiA11*) was supplied by G. Walker, Harvard University, Cambridge, USA.

Colicin production and immunity. L broth (Miller, 1972) and M63 minimal salts medium (Pardee *et al.*, 1959) containing 1% (v/v) glycerol and solidified with 1·6% or 0·6% agar were used. Patch tests were performed by

picking fresh colonies or growth of the test strain into a lawn seeded with 10^6 indicator cells in 3 ml soft agar (up to 100 test strains per plate). Zones of growth inhibition were scored after 8–24 h incubation at 37 °C. In the overlay test, an unseeded agar plate was stab inoculated with the test strain and incubated for 24 h at 37 °C. The plate was then inverted and a 4 cm square pad of Whatman no. 1 filter paper placed in the lid. The pad was then soaked with chloroform (approx. 0·3 ml) and the dish closed for 30 min. The surface was then overlaid with 3 ml soft agar containing the indicator strain and the plate reincubated. Care was exercised when using plastic Petri dishes because too much chloroform would cause the lid and dish to stick together. When glass or metal lids were used the paper pad was removed before the agar overlay was added; this was not necessary with plastic dishes. Cross-streak tests for colicin sensitivity were as described by Davies & Reeves (1975*b*).

Plasmids. These were extracted from cells by the methods of Holmes & Quigley (1981), which was ideal for small plasmids, or Kado & Liu (1981), which was ideal for large plasmids. Methods for transformation and conjugation with selection for colicin immunity or for antibiotic resistance encoded by the Col plasmid or by the Tra^+ mobilizing plasmid (see Table 1) were as used previously (Pugsley & Reeves, 1977; Mock & Pugsley, 1982). Agarose gel electrophoresis was as described by Maniatis *et al.* (1982) using Tris/acetate buffers in horizontal gels containing 0·5%, 0·8% or 1·2% agarose, depending on the size of the plasmid being examined. No attempt was made to determine the sizes of the Col plasmids other than to classify them as type I or type II (Lehrbach & Broda, 1984; Pugsley, 1984).

RESULTS AND DISCUSSION

Colicin/microcin producing strains

A collection of colicin-producing derivatives of *E. coli* K12 (Table 1) was obtained by transformation using plasmids extracted from the orginal producing strains, or by conjugation with the original strain with selection for immunity to the colicin produced by the donor strain. In cases where the original strain produced two colicins, selection was with a partially purified preparation of one only, or initially with a recipient which was sensitive to one only. The entire collection of strains was cross-checked for heterospecific immunity and, in two cases where the plasmid was found to confer immunity to a colicin other than the one encoded by the plasmid (pColE3-CA38 and pColIa-CA53), additional strains carrying *in vitro* constructed recombinant plasmids devoid of the additional immunity gene were added to the collection. The presence of a single plasmid of the appropriate size (Pugsley, 1984) was confirmed by agarose gel electrophoresis of plasmid extracts. Further details of the strains are given in Table 1 and in Pugsley (1984).

The list of colicins shown in Table 1 is not exhaustive. At least three other colicins have been described. Colicin L (bacteriocin JF246) is produced by *Serratia marcescens* strain JF246 (Foulds, 1972) and also apparently by *E. coli* 398 (Davies & Reeves, 1975*b*) and by three strains of *S. marcescens* recently isolated in our laboratory. In none of these cases was a plasmid detected in the producing strain or colicin immunity transferred to *E. coli* K12 by conjugation. *E. coli* strains CA46 and CA58 produce the related colicins G and H, respectively. Transfer of colicinogeny or colicin immunity from these strains to *E. coli* K12 was not achieved by conventional methods, even though the ColG and ColH plasmids have been tentatively identified (Lehrbach & Broda, 1984). Similarly, *Klebsiella pneumoniae* strain 492, which produces microcin E492, did not appear to carry a plasmid coding for the microcin or for immunity to it (V. de Lorenzo & A. P. Pugsley, unpublished observations). All of these strains, like those listed in Table 1, are available both from the author and from the PRC.

Conditions for colicin production

Production of most colicins is regulated by the SOS system (Pugsley, 1984), and therefore can be optimized by UV light irradiation or mitomycin C treatment of the Col^+ cells, or by using GW1000 [*recA441*, high level colicin production and SOS gene expression at 42 °C (Pugsley, 1981)] as the producing strain. Colicin production in plates was most conveniently tested by the patch or overlay methods (Methods). The former test had the advantage of requiring one cycle of incubation only, but had the disadvantage that inhibition zones were often small, particularly in the case of colicins B, H, Ia, Ib and M. The sensitivity of this test could be improved by including 0·1–0·25 μg mitomycin ml^{-1} in the agar. The sizes of the inhibition zones produced by the Col^+ strains could be increased by using strains carrying a chromosomal mutation which inactivated

the gene for the homologous colicin receptor protein (Tables 1 and 2). This was particularly effective with strains which produced very little colicin because the colicin which was released by the producing cells was largely resorbed onto the cell surface and was therefore unable to diffuse into the agar. Similarly, strains carrying *rfa* or *tolAB* mutations produced larger inhibition zones in plate tests, particularly if the strain produced small amounts of colicin. This seemed to be caused by the lysis of the colicin-producing cells within the colony (unpublished observation). Mutations inactivating colicin receptors were easily selected using the agents listed in Table 2, while strains carrying *rfa* could be constructed by transducing them to kanamycin resistance using P1 phage grown on strain PAP102 (*rfa*::Tn*5*). Such strains are not included in the collection available from PRC because the mutations affect the colicin sensitivity patterns of the producing bacteria, making them unsuitable for tests for colicin identity based on colicin immunity patterns (see below).

Production of colicin V, and of microcins C7, C17 (colicin X) and C492 was not induced by treatments which derepressed expression of SOS-regulated genes. Production of these antagonists was best observed using minimal glycerol medium rather than rich medium, and they could be further distinguished from other colicins by their ability to pass through cellophane membranes placed between the producing and indicator bacteria on the plate (Asensio *et al.*, 1976).

Colicin immunity and colicin-insensitive mutants

The specific colicin immunity of the *E. coli* K12 Col$^+$/Mcc$^+$ strains listed in Table 2 can be used as the basis of tests for the identification of new colicins. Some caution is required, however, because the strains may only be immune to low levels of colicin (Table 2). This was usually manifested by the decreased size of the inhibition zone rather than its complete absence when indicator strains are the same as the producing strains in patch, overlay or cross-streak tests. The occasional appearance of colicin-insensitive clones was noted in some of these 'partially immune' colicin-producing strains, most frequently in strains BZB2101 (ColA) and BZB2104 (ColE1), when the mutation was invariably of the *btuB* type (Mock & Pugsley, 1982). In addition, the formation of unusual inhibition zones with certain combinations of Col$^+$ indicator and producing bacteria was noted previously by Watson *et al.* (1981). The strains which produce this phenomenon produce colicins in the E2–E8 series, and the unusual inhibition zones may be caused by low level, non-specific cross-immunity.

Tests for colicin immunity could be supplemented by tests using specific colicin-insensitive indicator strains. Mutants of *E. coli* K12 which lack the ability to adsorb colicin (resistant) or which adsorb colicin but remain unaffected by it (tolerant) are listed in Table 2 together with the most suitable agents for use in their selection. All of these mutants lack one or more membrane proteins which are directly or indirectly implicated in colicin adsorption or transport into the cell (Pugsley, 1984). It should be noted that some of the insensitive mutants gave reactions of partial sensitivity on plate tests (Table 2).

Use of the strains collection for the identification of colicins

In the present context, the main use of the colicin strains collection is to assist in the identification of a new colicin, or to confirm the identity of a colicin already under investigation. The decreased sensitivity of Col$^+$ strains in Table 1 (relative to strain BZB1011) to an unidentified colicin is a good indication of the (partial) identity of the two colicins. Similarly, reduced activity against strains listed in Table 2 is also useful in the preliminary identification of a colicin. Two problems which can arise are first, more than one colicin may be produced by a Col$^+$ isolate, and second, Col plasmids listed in Table 1 may carry genes for the production of or immunity to as yet unidentified colicins (see Chak & James, 1984). The first problem can be overcome by the judicious use of indicator strains carrying combinations of different Col plasmids and/or colicin-insensitivity mutations. For example, a strain carrying both *btuB* and *tonB* mutations was insensitive to over 80% of colicins produced by a collection of natural Col$^+$ strains. Another approach is to select mutants which are insensitive to the colicin(s) produced by the uncharacterized Col$^+$ strain; double mutants occur very infrequently during a single

Table 1. *Colicin-producing derivatives of E. coli K12 W3110*

All strains are derivatives of *E. coli* K12 W3110; some of them carry a chromosomal *gyrA* mutation conferring resistance to nalidixate. Most plasmid designations indicate the strain from which the plasmid came (Reeves, 1972); plasmids isolated more recently in our laboratory, derivatives of Col plasmids carrying transposons, and *in vitro* constructed Col plasmids are given more conventional plasmid designations. Plasmids pAPBZ101 and pAPBZ106 are pBR322-carrying fragments derived from pColE3-CA38 and pColIa-CA53, respectively, cloned into the *Eco*RI restriction site (Mock & Pugsley, 1982; Pugsley, 1983). The former plasmid does not carry the ColE3-CA38 gene necessary for colicin release from producing cells (*celC*; Pugsley & Schwartz, 1983). Strain PAP222 carries pR538 in addition to pColV-K270 because we were unable to mobilize this Col plasmid by any other means. Microcin 17 and colicin X are probably identical; strains producing them are cross immune, both agents have a molecular weight of 5000, and they appear to have identical modes of action (M. Lavina and A. Pugsley, unpublished observations; Baquero & Moreno, 1984; Pugsley, 1984). Microcin designations are as used by de Lorenzo & Aguilar (1984). Further details are given in de Lorenzo & Aguilar (1984) and Pugsley (1984).

Strain no.	Plasmid	Colicin/ microcin produced	Colicin/ microcin immunity*	Plasmid type†	Conjugal plasmid transfer‡		Immediate source of Col/Mcc plasmid§
					Tra$^+$	Mob$^+$	
BZB2101	pColA-CA31	colA	(A-CA31) (A-23)	I	–	+	*Citrobacter freundii* CA31 (P. Fredericq via P. Reeves)
BZB2102	pColB-K260	colB	B-K260	II	+	NA	*E. coli* AG097 (B. Stocker)
BZB2103	pColD-CA23	colD	D-CA23	I	–	+	*E. coli* CA23 (P. Fredericq)
BZB2104	pColE1-K53	colE1	(E1-K53) (E1-K30)	I	–	+	*E. coli* K53 (P. Fredericq via P. Reeves)
BZB2125	pColE2-P9	colE2	E2-P9 E2-CA42	I	–	+	*E. coli* K12 (pColE2-P9) (D. Helinski via P. Reeves)
BZB2106	pColE3-CA38	colE3	E3-CA38 E8-J	I	–	+	*E. coli* CA38 (P. Fredericq via P. Reeves)

BZB2149	pAPBZ101 (Ap^R, Tc^R)	colE3	E3-CA38	I	–	–	Mock & Pugsley (1982)
BZB2107	pColE4-CT9	colE4	E4-CT9	I	–	+	*E. coli* 316BM (B. Stocker)
BZB2108	pColE5-099	colE5	E5-099	I	–	+	*E. coli* 217BM (B. Stocker)
BZB2109	pColE6-CT14	colE6	E6-CT14	I	–	+	*E. coli* 318BM (B. Stocker)
BZB2110	pColE7-K317	colE7	E7-K317	I	–	+	*E. coli* 245BM (B. Stocker)
PAP247	pPC101	colE8-J	E8-J	I	–	+	*E. coli* K12 (pPC101) (Cooper & James, 1984)
BZB2114	pColIa-CA53	colIa	(Ia-CA53) (A-CA31)	II	+	NA	*E. coli* CA53 (P. Fredericq via P. Reeves)
BZB2279	pAPBZ106 (Ap^R, Tc^R)	colIa	(Ia-CA53)	I	–	–	Pugsley (1983)
BZB2115	pColIb-P9 *drd*	colIb	(Ib-P9)	II	+	NA	*E. coli* K12 (pColIb-P9) (D. McCorquodale via P. Reeves)
BZB2116	pColK-K235	colK	(K-235) (K-216)	I	–	+	*E. coli* K235 (P. Fredericq via P. Reeves)
PAP1	pCHAP1 (ColM)	colM	M-CHAP1 M-K260	II	+	NA	*E. coli* BZBNC23 (new isolate, this laboratory)
BZB2123	pColN-284	colN	(N-284)	I	–	+	*E. coli* 284 (Y. Hamon via P. Reeves)
PAP2	pCHAP2 (ColS4)	colS4	S4-CHAP2 S4-P15	II	+	NA	*Salmonella typhimurium* S3409 (R. Barker)
PAP222	pColV-K270 (R538*drd*)	colV	V-K270 V-K30	II	–	+	*E. coli* K12 (pColV-K270 pR538*drd*) (P. Fredericq)
PAP54	pMM301 (pIP7006::Tn*5*)	mccC7	mccC7	II	+	NA	*E. coli* K12 (pMM301) (Baquero *et al.*, 1978 via F. Moreno)
BZB2283	pMM4 (pRYC17::Tn*10*)	mccB17	mccB17 X-CA23 X-W7	II	+	NA	*E. coli* K12 (pMM4) (Hernandez-Chico *et al.*, 1982)

NA, Not applicable.

* Parentheses indicate that immunity is overcome at high colicin concentrations.

† Plasmid types distinguished arbitrarily (Pugsley, 1983, 1984) on the basis of migration in agarose gels.

‡ Plasmids transferred spontaneously (Tra$^+$) or only in conjunction with pR64*drd*, pR538*drd* or F'*lac* Tn*10* Tn*9* Tn*5* (pAPBZ122) (Mob$^+$).

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Table 2. *Colicin/microcin-insensitive mutants of E. coli K12*

Preliminary data on which this table is based comes from Davies & Reeves (1975*a*, *b*).

Strain no.*	Mutation	Map (min)†	Selection	Resistance/tolerance‡																						
				Colicins																				Microcins		
				A	B	D	E1	E2	E3	E4	E5	E6	E7	E8	G	H	Ia	Ib	K	L	M	N	S4	V	X	C7	B17	E492
BZB1013	*fepA*	14	colD	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
BZB1030	*btuB*	90	phage BF23	(R)	S	S	(R)	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
BZB1022	*cir*	45	colIa	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	R/T	S	S	S	S
BZB1190	*tsx*	9	phage T6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
BZB1191	*tonA* (*fhuA*)	4	phage T5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
BZB1192	*tonB*	28	phage ϕ80 + colD	S	T	T	S	S	S	S	S	S	S	S	T	T	T	T	S	S	T	S	S	R/T	S	S	S	R/T
PAP308	*ompF*	21	colN (phage K20)	R	S	S	S	(T)	(T)	(T)	(T)	(T)	(T)	(T)	S	S	S	S	(T)	(T)	S	R	(R/T)	S	(R/T)	S	(R/T)	S
PAP138	*ompR*::Tn*5*	75	colN + phage PA2/λ.hy2 or TuIb (+K20)	R	S	S	S	T	T	T	T	T	T	T	S	S	S	S	T	T	S	R	R/T	S	R/T	S	R/T	S
PAP702	*ompA*	22	phage K3*h*1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	(T)	(T)	S	S	S	S	S	S	S	S
PAP709	*rcx*	9	mccB17	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R/T	S	R/T	S
PAP710	*rem*::Tn*5*	?	mccE472	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	(R/T)

* All strains are derivatives of W3110 or BZB1011.

† Map positions are those given by Bachmann (1983) except those for *cir* (Boos *et al.*, 1983) and *rcx* (M. Lavina and F. Moreno, personal communication), and were confirmed for the present mutations by phage P1-mediated co-transduction with appropriate mutations (Bachmann, 1983). The map location of *rem* has not been determined.

‡ S, sensitive; T, tolerant; R, resistant; R/T, mechanism of insensitivity unknown; parentheses, partial reaction.

selection cycle but the isolation of such mutants having characteristics of two classes of mutants listed in Table 2 is a good indication of the presence of two colicins. The problem of unidentified immunity genes can be overcome by transferring the Col plasmid into *E. coli* K12 (e.g. BZB1011) and then performing a reciprocal colicin immunity test using strains listed in Table 1.

The shared identity of two colicins can only be inferred rather than confirmed by the above procedures. Positive identification depends on other techniques, including shared modes of action and physical and chemical homology (including serological and amino acid sequence homology). The possible existence of microheterogeneity among colicins of a given type has not been investigated, but colicins which are apparently indistinguishable may be encoded by plasmids which are seemingly quite different [e.g. colicin E2 (Watson *et al.*, 1983) and colicin N (unpublished observations)]. Indeed, the apparent high degree of homology of colicins E2 to E8 may represent one example of microheterogeneity among closely related colicins, although in this case the colicins can be distinguished by the absence of significant cross-immunity, differences in modes of action, and incomplete serological homology (Mock & Pugsley, 1982).

The collection of strains described here has been used by us to identify new types of colicins or microcins. The initial stage was to plate samples of water, soil and other materials on MacConkey lactose agar, and to test Lac$^+$ clones which developed at 37 °C for the production of an inhibitor active against strain BZB1011 in patch tests on L agar + mitomycin or on M63 glycerol agar. Over 35% of 10000 colonies screened produced such an inhibitor. The producing clones were purified from the patch plate, and tested for activity against strain PAP15 (*btuB tonA cir tsx fepA*): 70% of the strains produced an inhibitor which was completely inactive against this strain, and a further 12% had substantially reduced activity. All but 35 of the remaining strains were inactive when tested against strain PAP15 carrying a *tonB* mutation. Eight of these 35 strains produced an inhibitor which had reduced activity against strains carrying *ompF* or *ompR* mutations, or pCHAP2 (ColS4), and produced inhibition zones which were similar to those produced by strains producing colicin S4 (large zones with high background growth of indicator strain). The remaining 27 strains were assumed to produce new colicins or microcins, possibly in combination with colicins which have been characterized. These results illustrate the utility of the strains in the collection, and indicate that yet more colicins or microcins are still to be identified and characterized.

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