Molecular Comparisons of Plasmids Isolated from Colicinogenic Strains of
Escherichia coli

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The plasmid content of 14 colicinogenic strains of Escherichia coli has been examined. Four strains contained miniplasmids (1-2-2.0 kb). Small plasmids (4-7 kb) were detected in all the strains specifying group A colicins (colicins A, E1, E2, E3 and K; group I plasmids). Larger plasmids (55-130 kb) were detected in seven out of nine strains specifying group B colicins (colicins B, H, Ia, Ib, M, V and S1; group II plasmids). DNA–DNA hybridization with group II plasmids showed a wide variation in the degree of DNA sequence homology among its members. In contrast little (if any) DNA sequence homology was detected with the group I plasmids when the same group II plasmid DNAs were used as hybridization probes. The results of DNA–DNA hybridization studies with the two small group II plasmids (pcolG-CA46 and pcolD-CA23) suggested that these plasmids are equivalent to deleted forms of larger group II plasmids. Our hybridization data thus support the division of colicin plasmids into the two groups (I and II) that have been previously defined from genetic and physiological studies.

INTRODUCTION

Plasmids of Escherichia coli that determine the synthesis of antibacterial proteins, called colicins, have been classified into two major taxonomic groups, I and II (Hardy et al., 1973). Groups I (colicins E1, E1a, E2 and K) and II (colicins B, Ia, Ib and V) were defined by a number of physiological criteria such as the effect of the host recA allele on colicin titres and the mean number of copies of the colicin factors per chromosome (Hardy et al., 1973). Support for these groupings was provided by a subsequent study of the host range of colicins on a series of colicin-resistant mutants of E. coli (Davies & Reeves, 1975a, b). Mutants selected as being resistant to colicins of their group A (colicins A, E1, E2, E3, K, L, N, S4 and X) might or might not also be resistant to other colicins of the same group but they were never resistant to colicins of group B (colicins B, D, G, H, Ia, Ib, M, Q, S1 and V). Similarly the mutants resistant to a group B colicin could be resistant to others in this group, but were never resistant to group A colicins. Hence colicin factors of groups I and II appear to specify colicin proteins of groups A and B, respectively.

In general the groups also differ in the molecular weights of the plasmids specifying the colicin proteins (Hardy et al., 1973; Hughes et al., 1978). An examination of the plasmids found in 26 colicinogenic strains, by rate zonal centrifugation in sucrose–ethidium bromide gradients and by electron microscopy, suggested that plasmids specifying colicins of group A had evolved from a small ‘EK-like’ ancestor with a molecular weight of about 5 × 10^6 (approximately 7.5 kb) whereas the plasmids specifying colicins of group B were descended from a larger ‘BIV-like’ ancestor with a molecular weight of 40 × 10^6–70 × 10^6 (60–100 kb). Some exceptions to this

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Abbreviations: CCC, covalently closed circular; OC, open circular.

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generalization were, however, noted (Hughes et al., 1978) since large group I plasmids (ColL) and small group II plasmids (ColD and ColG) were also found.

In this study we have investigated the possible evolutionary relationships between these groups of colicin plasmids by determining the extent to which plasmid DNAs, isolated from colicinogenic bacteria, share common DNA sequences. Using restriction endonuclease analysis and DNA-DNA hybridization we have characterized these plasmids and then examined the relationships between several of the group II plasmids.

METHODS

**Bacterial strains.** Colicinogenic strains are listed in Table 1. All colicinogenic strains were kindly provided by Dr K. G. Hardy.

**Media.** Media and culture conditions were as previously described (Duggleby et al., 1977).

**Purification of plasmid DNA and restriction endonuclease analyses.** Plasmid DNA was prepared by a cleared lysate method (Guerry et al., 1973) or by a procedure based on the method of Hansen & Olsen (1978) and purified following CsCl-ethidium bromide density gradient centrifugation.

Restriction endonucleases HindIII (BRL) and EcoRI (Boehringer-Mannheim) were used as specified by the manufacturers. PstI and SalI were prepared in this laboratory by L. Wallace, J. Ward and P. Lehrbach and used as specified in the BRL catalogue.

### Table 1. Plasmid content of colicinogenic strains

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Colicin produced†</th>
<th>Source and/or reference</th>
<th>Plasmid and size (kb)‡</th>
</tr>
</thead>
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<tr>
<td>M1242, <em>E. coli</em> 23</td>
<td>A-23 (A)</td>
<td>K. Hardy</td>
<td>pcolA-23, 4.3; pMT061, 2.0; pMT062, 1.2</td>
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<tr>
<td>KH753, <em>E. coli</em> K12</td>
<td>E1-K30 (A)</td>
<td>Davies et al. (1981)</td>
<td>pcolE1-K30, 6.3</td>
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<td>W3110 (colEI-K30)</td>
<td>E2-P9 (A)</td>
<td>Davies et al. (1981)</td>
<td>pcolE2-P9, 6.6</td>
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<tr>
<td>KH293, <em>E. coli</em> K12</td>
<td>E3-CA38 (A)</td>
<td>K. Hardy</td>
<td>pcolE3-CA38, 6.8; (&gt;70)</td>
</tr>
<tr>
<td>AB1157 (colE2-P9)</td>
<td>E-J2 (A)</td>
<td>Davies &amp; Reeves (1975b)</td>
<td>pcolK-J2, 6.8; pLG500, 1.3</td>
</tr>
<tr>
<td>KH28, <em>E. coli</em> CA38 (colcinI- derivative)</td>
<td>E-J2 (A)</td>
<td>Broome-Smith (1980)</td>
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<tr>
<td>M1247, <em>E. coli</em> K216</td>
<td>K-K216 (A)</td>
<td>Davies &amp; Reeves (1975b)</td>
<td>pcolK-K216, 6.8; pLG500, 1.3</td>
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<tr>
<td>KH142, <em>E. coli</em> K77</td>
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<td>Hardy et al. (1973)</td>
<td>pcolB-K77, 85</td>
</tr>
<tr>
<td>KH1047, <em>E. coli</em> CA23</td>
<td>D-CA23 (B)</td>
<td>K. Hardy</td>
<td>pcolD-CA23, 5.0 (&gt;30)</td>
</tr>
<tr>
<td>KH1046, <em>E. coli</em> CA46</td>
<td>X-CA23 (A)</td>
<td>Davies &amp; Reeves (1975b)</td>
<td></td>
</tr>
<tr>
<td>CL129, <em>E. coli</em> CA58</td>
<td>G-CA46 (B)</td>
<td>K. Hardy</td>
<td>pcolG-CA46, 4.6</td>
</tr>
<tr>
<td>M1231, <em>E. coli</em> CA53</td>
<td>H-CA58 (B)</td>
<td>Hughes et al. (1978)</td>
<td>pcolH-CA58, 94; pMT063, 1.2</td>
</tr>
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<td>KH596, <em>E. coli</em> K12</td>
<td>Ia-CA53 (B)</td>
<td>Hughes et al. (1978)</td>
<td>pcolIa-CA53, 120</td>
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<tr>
<td>J5-3 (colIb-P9)</td>
<td>Ib-P9 (B)</td>
<td>Hughes et al. (1978)</td>
<td>pcolIb-P9, 86</td>
</tr>
<tr>
<td>KH273, <em>E. coli</em> K12 (colM-K260)</td>
<td>M-K260 (B)</td>
<td>K. Hardy</td>
<td>pcolM-K260, 55</td>
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<td>M1240, <em>E. coli</em> CA7</td>
<td>V-CA7 (B)</td>
<td>Hughes et al. (1978)</td>
<td>pcolV-CA7, 78</td>
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<tr>
<td>CL15, <em>E. coli</em> (colSI-P1)</td>
<td>S1-P1 (B)</td>
<td>K. Hardy</td>
<td>pcolSI-P1, 130; pMT064, 1.9</td>
</tr>
</tbody>
</table>

* Escherichia coli 23 produces a second unidentified colicin (Davies & Reeves, 1975b). Escherichia coli CA7 produces colicin V and one other colicin of group B (Davies & Reeves, 1975a). KH273 is an *E. coli* K12 strain harbouring a Tra+, colicin M-specifying plasmid that also carries genes for the synthesis of cysteine and tryptophan (K. Hardy, personal communication). CL15 is an *E. coli* strain harbouring a colicin S1-specifying plasmid transferred from *Shigella boydii* P1 (K. Hardy, personal communication).

† The colicin group is given in parentheses.

‡ Plasmid DNA was prepared from strains M1242, M1247, KH28, KH293, KH573, KH1046 and KH1047 by the cleared lysate method (Guerry et al., 1973) and from the remaining strains by a procedure based on that of Hansen & Olsen (1978). Plasmid DNA sizes were determined by agarose gel electrophoresis of fragments obtained by cleavage with restriction endonucleases EcoRI, HindIII, PstI and SalI and by comparison with known DNA size markers (see Methods). Nomenclature for colicin plasmids follows the recommendations of Reeves (1972); it indicates the colicin produced and the strain from which the plasmid was originally derived. Other plasmid species were named in this laboratory, except pLG500 (Broome-Smith, 1980). Plasmid DNA sizes in parentheses indicate plasmid species (from strains KH28 and KH1047) detected in only low yields; no attempt was made to characterize these plasmids further.
Digested plasmid DNA was analysed by electrophoresis in 0.5%, 0.7%, or 1% (w/v) agarose gels with a horizontal gel apparatus. The following plasmids and phage DNA of known molecular size were used as markers: RP4, 56.5 kb (Meyer et al., 1977); pWW0 (TOL), 115 kb (Lehrbach et al., 1982); λ, 48.5 kb (Mayer et al., 1981); R2, 73 kb (Bayley et al., 1979); and pBR322, 4.3 kb (Bolivar et al., 1977). Bacteriophage λ digested with HindIII and pWW0 DNA digested with HindIII or XhoI (Downing & Broda, 1979) were also used as DNA fragment size markers.

**DNA--DNA hybridization in paraffin oil.** A measure of the DNA sequence homology between plasmid DNA species was determined essentially as described by Roussel & Chabbert (1978) except that plasmid DNA was labelled with 32P by nick-translation (see below) and whole-cell DNA was isolated by the method of Dhaese et al. (1979). 32P-labelled sonicated plasmid DNA [106 c.p.m. (μg DNA)−1] was heat denatured and used to probe an excess of unlabelled single stranded whole-cell DNA bound to nitrocellulose filters (100 μg DNA per 25 mm diam. filter). Hybridizations were performed on microfilters punched from the large DNA-loaded filters (8 x 5 mm diam. filters per 25 mm diam. filter; 2000 c.p.m. 32P-labelled plasmid DNA per filter). Hybridizations were performed on microfilters punched from the large DNA-loaded filters (8 x 5 mm diam. filters per 25 mm diam. filter; 2000 c.p.m. 32P-labelled plasmid DNA per 25 mm diam. filter) at 37 °C for 18 h in 2 x SSC (1 x SSC = 0.15 M-NaCl, 15 mM-sodium citrate, pH 7.0), 50% (v/v) formamide under paraffin oil as described by Roussel & Chabbert (1978). The microfilters were then washed in 2 x SSC before drying and the amount of 32P-labelled DNA bound to each microfilter was determined by a 10 min count in a liquid scintillation spectrophotometer. Sequence homology is expressed as the percentage of reassociation of 32P-labelled sonicated and heat-treated plasmid DNA with unlabelled single-stranded whole cell DNA from strains carrying homologous or heterologous plasmids. Data (Table 2) are the mean of four separate determinations and are expressed relative to the reassociation with DNA of the same plasmid ( = 100) and with DNA of a plasmid-free host strain ( = 0).

**Transfer of DNA to nitrocellulose filters.** DNA restriction fragments separated by agarose gel electrophoresis were transferred to nitrocellulose filters (Schleicher & Schuell BA85) by the method of Southern (1975).

**Preparation of 32P-labelled plasmid DNA and DNA--DNA hybridization conditions.** Plasmid DNA (approximately 1 μg) was 32P-labelled by nick-translation essentially as described by Rigby et al. (1977) using [32P]dATP as the labelled precursor. After 4 h incubation the unreacted triphosphates were removed by passing the mixture through a Sephadex G-50 column. Nitrocellulose filters containing the DNA fragments were wetted with 2 x SSC, 0.1% (w/v) SDS and 50% (v/v) formamide for 10 min at 37 °C in confined plastic bags. Excess fluid was then removed.

32P-labelled plasmid DNA, sonicated to an average size of 500 bp and denatured by boiling for 10 min, was added to the filters. The plastic bags were then sealed and the hybridizations carried out at 37 °C for 16 h. After hybridization the filters were washed twice in 2 x SSC, 0.1% SDS at 37 °C and then twice in 2 x SSC (room temperature). For autoradiography, dried filters were exposed to sensitized photographic film next to an intensification screen at −70 °C. Autoradiography was carried out for 24 h, and then for a further 3–6 d to reveal weakly hybridizing bands.

**RESULTS**

**Plasmid content of colicinogenic strains**

Fourteen strains were chosen to represent those producing individual colicin types within the two major taxonomic groups (Table 1). Extracts containing their CCC DNA species were prepared and analysed by agarose gel electrophoresis. For the larger plasmids (> 50 kb), size estimates were confirmed by adding the molecular sizes of DNA fragments generated by restriction endonuclease digestion. Plasmid DNA was detected in all the strains examined; several strains contained more than one plasmid species (Fig. 1 and Table 1). M1242 (E. coli 23) had the most, with three species; of these the 4.3 kb colicin A plasmid (pcolA-23) is similar in size to a previously reported colicin A plasmid (pcolA-CA31, 4.2 kb) (Mock & Pugsley, 1982). The other plasmids from E. coli 23, pMT061 and pMT062 (2.0 and 1.2 kb, respectively), have not been previously reported explicitly. However, Hughes et al. (1978), using electron microscopy, observed a mixed population of plasmids in E. coli 23. Minitransplants were also found in three other cases, strains M1247, CL15 and CL129. By size comparisons with the well characterized miniplasmid pLG500 from E. coli K12 (Broome-Smith, 1980) (i.e. M1247) the sizes of the miniplasmids isolated from CL15 and CL129 (pMT064 and pMT063) were estimated to be 1.9 and 1.2 kb, respectively.

All representatives of the strains producing group A colicins (that is, colicins A, E1, E2, E3 and K) contained small plasmids of between 4 and 7 kb, confirming the observations of Inselburg (1974) and Hughes et al. (1978) but these plasmids were not analysed further here.
Fig. 1. (a) 0.7% agarose gel electrophoresis of plasmid DNA samples from: 1, M1242; 2, M1247; 3, KH1046; 4, KH1047; 5, CL129; and 6, CL15. (b) Schematic representation. The mobilities of OC and CCC (+) forms of the various plasmid species were determined by analysing DNA samples before and after controlled pancreatic DNAase nicking or treatment with restriction endonucleases. The plasmid(s) were identified in the strains as follows: M1242 (pcolA-23, OC-1 and CCC-1*; pMT061, 2 and 2*; pMT062, 3 and 3*), M1247 (pcolK-K216, 1 and 1*; pLG500, 2 and 2*), KH1046 (pcolG-CA46, 1 and 1*), KH1047 (pcolD-CA23, 1*), CL129 (pMT063, 1 and 1*), and CL15 (pMT064, 1 and 1*). Contaminating chromosomal DNA (chr) runs as a single faint band.

Recently the observation that plasmids specifying group A colicins are of low molecular weight has been extended to other newly recognized colicin E-producing strains (Watson et al., 1981; Mock & Pugsley, 1982).

Among the strains producing group B colicins, small plasmids were also found in strains KH1046 (pcolG-CA46, 4-6 kb) and KH1047 (pcolD-CA23, 5-0 kb). These plasmids are discussed in a later section. The remaining group B colicin strains contained larger plasmids. The sizes of restriction fragments of plasmids isolated from seven strains (KH142, KH273, KH596, M1231, M1240, CL15 and CL129) were determined (data not shown).

On the basis that only related plasmids yield similar restriction endonuclease cleavage fragments these plasmids are only distantly related. For instance with the restriction endonucleases HindIII and SalI (which give between 6–12 and 1–21 fragments, respectively), the seven plasmids yield few common bands. Where fragment size analogues between two plasmids are observed (for instance with PstI restriction fragments) these fragments are usually <10 kb. Even the plasmids belonging to the same incompatibility groups did not have common sized fragments; for instance pcolIb-P9 and pcolIa-CA53 (both IncIa) and pcolV-CA7 and pcolB-K77 (both IncFII; Jacob et al., 1977) show few common bands.
DNA hybridization with colicin plasmids

DNA reassociation studies

A more sensitive test of plasmid relatedness is DNA hybridization at high stringency on nitrocellulose microfilters using $^{32}$P-labelled DNA (Roussel & Chabbert, 1978; Bayley et al., 1979). Plasmids of group II (except pcolM-K260) were examined, and their relatedness to representatives of the group I plasmids was also tested (Table 2). The range of homologies between the seven large group II plasmids (pcolB-K77, pcolIb-P9, pcolIa-CA53, pcolIV-CA7, pcolSI-P1, pcolM-K260 and pcolH-CA58) was 3–51%. An examination of reciprocal hybridizations presented in Table 2 shows that in most cases (21/28), reciprocal experiments yield a similar size estimate for common DNA sequences. In the remaining cases however there were some apparent inconsistencies. For example reciprocal experiments with $^{32}$P-labelled pcolIb-P9 and pcolIa-CA53 DNA gives the size of common DNA sequences as 44 kb and 57 kb, respectively (Table 2). At present we are unable to explain these differences but in some cases they may reflect the nature of specific sequence relationships between particular plasmids. These differences may also have resulted from using a mixture of $^{32}$P-labelled plasmid species as hybridization probes (for example strains CL15, CL129 and KH1047). Further experiments are therefore needed to examine and resolve these differences.

In contrast to the range of homologies within the group II colicin plasmids there was little or no hybridization and therefore no relationship with the group I plasmids. For the isolated cases where some homology was detected, these results are most likely explained by homology with larger molecular weight plasmids resident in the group I strain tested (KH28 with $^{32}$P-labelled pcolIb-P9 and pcolIa-CA53 DNA) or homology between strains harbouring miniplasmids (M1247 with $^{32}$P-labelled pMT063 and pMT064).

Homology among plasmids specifying colicins H, G, D and B

An exception to the rule that only large plasmids specify group B colicins (i.e. group II plasmids) are the small plasmids which determine colicins D and G (Table 1). Colicin D is very similar to colicin B (Davies & Reeves, 1975a; Pugsley & Reeves. 1977) and colicin G is similar to colicin H (Hughes et al., 1978; Davies & Reeves, 1975a), so that the plasmids encoding colicins D and G may have arisen by extensive deletion of the colicin B and H plasmids, respectively. These plasmids are indeed related since $^{32}$P-labelled plasmid DNA from KH1047 (pcolD-CA23) hybridizes with DNA from the colicin B-producing strain KH142 and $^{32}$P-labelled plasmid DNA pcolG-CA46 hybridizes with DNA of the colicin H-producing strain CL129 (Table 2).

The related DNA segments were localized in hybridizations using $^{32}$P-labelled probe DNAs of the four colicin plasmids and Southern blots of restriction endonuclease-generated fragments of unlabelled plasmid DNA preparations (Figs 2 and 3). This analysis was complicated by the fact that strain KH1047 contains two plasmid species, pcolD-CA23 (5 kb) and an uncharacterized high molecular weight plasmid (Table 1). To avoid DNA restriction fragments from the larger plasmid interfering with the hybridization analysis, unrestricted plasmid DNA samples were used; therefore both OC and CCC forms of pcolD-CA23 are present (Fig. 2; see also Fig. 1).

The hybridization patterns of pcolB-K77 (digested with EcoRI), undigested pcolD-CA23, pcolG-CA46 (digested with PstI) and pcolH-CA58 (digested with EcoRI) using the four different plasmid DNAs as $^{32}$P-labelled DNA probes are shown in Fig. 3. pcolB-K77 DNA (Fig. 3a) hybridized to all the EcoRI digestion fragments of pcolB-K77 (the control, lane 2) and the OC and CCC forms of pcolD-CA23 (lane 3). For the reciprocal experiment, $^{32}$P-labelled DNA isolated from KH1047 (containing pcolD-CA23) (Fig. 3c) hybridized to a single 5.6 kb pcolB-K77 EcoRI fragment. In an analogous manner, $^{32}$P-labelled pcolH-CA58 (Fig. 3b) hybridized to pcolG-CA46 (lane 4) and in the reciprocal experiment pcolG-CA46 (Fig. 3d) hybridized to a single EcoRI fragment (6.3 kb) of pcolH-CA58 (lane 5). These data indicate that in each case the relationship between the small plasmid and the large one resides on the large one in a discrete segment.
Table 2. DNA reassociation of the colicin plasmids

<table>
<thead>
<tr>
<th>Plasmid-carrying strain from which unlabelled total DNA was prepared*</th>
<th>KH142 colB-K77</th>
<th>KH596 colIb-P9</th>
<th>M1231 colIa-CA53</th>
<th>M1240 colV-CA7</th>
<th>CL15 colS1-P1</th>
<th>CL129 colH-CA58</th>
<th>KH1046 colG-CA46</th>
<th>KH1047 colD-CA23, colX-CA23</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH142 (II)</td>
<td>100</td>
<td>28 (24)</td>
<td>10 (12)</td>
<td>30 (23)</td>
<td>12 (16)</td>
<td>13 (12)</td>
<td>0</td>
<td>32 (2)</td>
</tr>
<tr>
<td>KH596 (II)</td>
<td>20 (17)</td>
<td>100</td>
<td>48 (57)</td>
<td>8 (6)</td>
<td>27 (35)</td>
<td>3 (3)</td>
<td>0</td>
<td>0</td>
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<td>M1231 (II)</td>
<td>11 (9)</td>
<td>51 (44)</td>
<td>100</td>
<td>25 (20)</td>
<td>23 (30)</td>
<td>12 (11)</td>
<td>8 (0.5)</td>
<td>0</td>
</tr>
<tr>
<td>KH273 (II)</td>
<td>18 (15)</td>
<td>30 (26)</td>
<td>6 (7)</td>
<td>20 (16)</td>
<td>6 (8)</td>
<td>22 (20)</td>
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<td>M1240 (II)</td>
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<td>18 (15)</td>
<td>6 (7)</td>
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<td>8 (10)</td>
<td>5 (5)</td>
<td>60 (3)</td>
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<td>CL15 (II)</td>
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<td>21 (25)</td>
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<td>CL129 (II)</td>
<td>12 (10)</td>
<td>3 (3)</td>
<td>14 (17)</td>
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<td>2 (1.5)</td>
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<td>4 (5)</td>
<td>10 (9)</td>
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<td>KH28 (I)</td>
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</table>

* The colicin plasmid group is given in parentheses.
† Values shown are for the reassociation of 32P-labelled plasmid DNA with excess unlabelled whole-cell DNA. Data are the mean of four separate determinations after deduction of the values from control hybridization experiments. The control values were obtained using 32P-labelled plasmid DNAs hybridized to blank filters and to whole cell DNA from the E. coli K12 plasmid-free strain ED8654. These values were <1% and 5-8% (respectively) of those obtained with hybridizations to whole-cell DNA from strains carrying homologous plasmids. Values in parentheses are the calculated sizes (kb) of common DNA sequences between two plasmids (plasmid size × percentage reassociation value).
DNA hybridization with colicin plasmids

Fig. 2. (a) Restriction patterns of isolated plasmid DNA obtained by 0.7% agarose gel electrophoresis. 1, pWW0 (HindIII-restricted); 2, pcolB-K77 (EcoRI); 3, pcolD-CA23 (unrestricted); 4, pcolG-CA46 (PstI); 5, pcolH-CA58, pMT063 (EcoRI); and 6, λ DNA (HindIII). (b) Schematic representation. Note that pMT063 is not cut with EcoRI, so that both OC and CCC plasmid DNA forms are present. pWW0 (TOL) DNA (Downing & Broda, 1979) and λ DNA restricted with HindIII were used as DNA size markers (kb).

DISCUSSION

We have examined the molecular relationships between plasmid DNAs isolated from 14 colicinogenic strains of E. coli. Earlier studies have examined the relationships between several of the small molecular weight plasmids isolated from strains specifying group A colicins (Inselburg, 1973, 1974; Inselburg & Johns, 1975; Watson et al., 1981); our studies were concerned with the plasmids isolated from strains specifying group B colicins and their relationship to each other and to the group A-specifying plasmids.

A comparison of restriction patterns of seven large molecular weight group II plasmids showed few DNA fragment sizes in common (data not shown) and therefore lack of a close relationship (Thompson et al., 1974). However DNA–DNA microfilter hybridizations showed a range of DNA reassociation values within this group of plasmids ranging from slight to extensive (Table 2). We conclude that these colicin plasmids may have a common origin, but that the genomes have diverged more or less extensively. This divergence is sufficient to prevent the convincing use of the restriction endonuclease method to show a relationship. Our DNA–DNA hybridization data therefore support the proposals of Hardy et al. (1973), Davies & Reeves (1975a, b), Hardy (1976) and Hughes et al. (1978). We also suggest that any relationship between the two groups is very distant.

The eight group II plasmids tested show different degrees of relationships; some related sequences may encode common transfer and/or incompatibility functions, as with the IncI plasmids pcolIa-CA53 and pcolIb-P9 or the IncFII plasmids pcolB-K77 and pcolV-CA7. They
Fig. 3. Southern blot hybridizations of $^{32}$P-labelled plasmid DNA. Experiments with $^{32}$P-labelled plasmid probe DNA: (a) pcolB-K77; (b) pcolH-CA58, pMT063; (c) pcolD-CA23; and (d) pcolG-CA46. Hybridizations were to filters containing plasmid DNA treated and arranged as shown in Fig. 2. No hybridization to the size marker DNA fragments (pWW0 or λ) was observed and these lanes (1 and 6) have therefore been excluded from the autoradiographs.
DNA hybridization with colicin plasmids

could also reflect repeated replicon fusion events similar to those detected in vivo between IncN and IncW plasmids (Nugent & Hedges, 1979) or postulated to have occurred in the evolution of the IncF1 plasmid group (Bergquist et al., 1982).

Microfilter and Southern blot DNA hybridizations were also used to investigate the relationships of the group II small plasmids pcolD-CA23 (5.0 kb) and pcolG-CA46 (4.6 kb). We find, as predicted by Hughes et al. (1978), that pcolD-CA23 DNA only hybridizes to a single EcoRI fragment (5.6 kb) of the colicin B plasmid pcolB-K77, and pcolH-CA58 DNA only hybridizes to a single EcoRI fragment (6.5 kb) of the colicin H plasmid pcolH-CA58.

We have not attempted to localize the homologous regions of the other group II plasmids or determine whether common 'core sequences' exist. The data from any such study would be difficult to analyse in the absence of a well-characterized reference plasmid. The recent genetic and physical analyses of the colIb-P9 plasmid (Boulnois, 1981; Boulnois et al., 1982), a plasmid included in this study, might provide such a point of reference.

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REFERENCES


pWW0 and pND2 and various R-plasmid-TOL derivatives from *Pseudomonas*. *Journal of Bacteriology* 152, 1280–1283.


