Escherichia coli ColV plasmid pRK100: genetic organization, stability and conjugal transfer

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Uropathogenic Escherichia coli strains express chromosomal and plasmid-encoded virulence-associated factors such as specific adhesins, toxins and iron-uptake systems. A ColV plasmid (pRK100) of a uropathogenic strain and its host KS533 were studied. The host strain encodes the K1 capsule, and P and S fimbriae, but neither haemolysin nor the cytotoxic-necrotic factor CNF1, indicating that this strain does not harbour a larger pathogenicity island. A restriction map of pRK100 was constructed on the basis of hybridization experiments and nucleotide sequencing. pRK100 harbours ColV, the conserved replication region RepFIB, the aerobactin-uptake system, a RepFlC replicon and additionally Colla as well as transposon Tn5431. The location of the RepFIC replicon was similar to that in plasmid F. ColV plasmids and F thus share a region spanning more than half the length of plasmid F. Even though their replication and transfer regions are homologous, ColV plasmids are found only in E. coli strains. Among the four other species tested, conjugal transfer of pRK100 was demonstrated, with low frequency, only to Klebsiella pneumoniae, suggesting that a natural barrier effectively bars transfer. In vitro stability of the plasmid with integration into the chromosome to ensure maintenance in the presence of an incompatible plasmid was demonstrated.

Keywords: ColV plasmid, plasmid pRK100, stability, conjugal transfer

INTRODUCTION

Pathogenic strains of Escherichia coli can cause intestinal and extraintestinal infections as well as newborn meningitis (Orskov & Ørskov, 1992). They encode various virulence determinants—adhesins, toxins, capsules, invasins and other virulence factors—on the chromosome, on plasmids, or on the genomes of bacteriophages. Virulence genes can be organized into large chromosomal blocks termed pathogenicity islands (Blum et al., 1995).

Plasmids are self-replicating and are normally not essential for bacterial growth; however, they often encode antibiotic resistances, colicins and various virulence determinants. Conjugative plasmids, in particular, facilitate the exchange and spread of resistances to antibiotics and chemicals, virulence factors and metabolic properties. The ColV plasmids form a heterogeneous group of large plasmids belonging to the IncF1 incompatibility group and encoding the production of colicin V (Waters & Crosa, 1991). ColV plasmids are harboured primarily by virulent enteric bacteria and encode several virulence-associated properties. Colicin V is a small molecule, but is not SOS inducible and is therefore actually a microcin (Braun et al., 1994).

Recent studies in a chicken embryo model system have shown that an avirulent wild-type avian E. coli strain transformed with the cloned colicin V genes became virulent, demonstrating that colicin V has a direct role in virulence enhancement (Wooley et al., 1994). Most colicin V plasmids also encode the aerobactin-uptake system and increased serum survival as well as resistance to phagocytosis. They could serve as models for the study of the evolution and molecular biology of other virulence plasmids.

ColV plasmids can harbour more than one replicon. The main replicon is homologous to the RepFIB of plasmid F. This replication region, along with the aerobactin-uptake system, is highly conserved. Some ColV plasmids also carry a replicon homologous to RepFIA of plasmid F and it has been reported that pColV-K30, the
prototypic ColV plasmid, also carries an incomplete RepFIC (Waters & Crosa, 1991) even though its location has not been determined.

Colicin V production was first described more than 70 years ago (Gratia, 1925), indicating that ColV plasmids were present in natural E. coli populations well before the widespread use of antibiotics. Use of antibiotics has probably selected for carriage of additional virulence and resistance determinants on ColV plasmids.

pRK100 is an approximately 145 kb conjugative plasmid which was discovered in a uropathogenic E. coli strain (Zgur-Bertok & Grabnar, 1990). The plasmid encodes colicins V and Ia, the aerobactin-uptake system and the 16:1 kb transposon Tn5431 with ampicillin and tetracycline resistance determinants (Zgur-Bertok et al., 1994, 1996). The present work was carried out to study pRK100, to prepare its map, and to gain insight into its evolution, conjugal transfer and stability.

METHODS

Bacterial strains, plasmids and media. The E. coli strains and plasmids used in this study are listed in Table 1. K5333 is a uropathogenic strain, serotype rough: K1H7 (determined by I. Oršković), isolated at the Institute of Microbiology, Medical Faculty of Ljubljana. It harbours a large (145 kb) conjugative plasmid, pRK100 (Zgur-Bertok & Grabnar, 1990). E. coli HB101 was used as the recipient strain for the conjugal transfer of plasmids pRK100 and RSF2001, a kanamycin-resistant derivative of plasmid F. E. coli DH5α was used as recipient for recombinant plasmids.

Plasmid pColVK-30, whose map has already been published (Waters et al., 1989), was used for comparison of probe binding in the hybridization experiments for IS1, RepFIA, RepFIB, RepFIC and RepFII, the aerobactin-uptake system and the tra region. Plasmids of the Couturier bank of rep probes were used for replication typing.

The Klebsiella pneumoniae, Enterobacter cloacae and Pseudomonas aeruginosa strains used as recipients in conjugation experiments are clinical isolates. The Salmonella typhimurium recipient was LT2.

Strains were grown in Luria–Bertani (LB) medium. When necessary, LB was supplemented with: 10 μg ampicillin ml⁻¹ (Ap); 10 μg tetracycline ml⁻¹ (Tc); 100 μg streptomycin ml⁻¹ (Sm); 10 μg chloramphenicol ml⁻¹ (Cm); 30 μg kanamycin ml⁻¹ (Kn). For selection of S. typhimurium, K. pneumoniae, E. cloacae, P. aeruginosa transconjugants, Simmons citrate media (Collee & Miles, 1989) supplemented with the appropriate antibiotics was used.

General DNA manipulation techniques. Prior to DNA manipulation, plasmids pRK100 and pColVK-30 were transferred to E. coli HB101. Isolation of plasmid and chromosomal DNA, large-scale isolation of CsCl-purified pRK100, and ligation and transformation experiments were performed as described by Sambrook et al. (1989). To visualize shorter restriction fragments pRK100 DNA was concentrated by ultrafiltration through a Centricon concentrator (Amicon) prior to restriction enzyme cleavage. Plasmids pRK100 and pColVK-30 were cleaved with EcoRI, HindIII, SalI, XhoI and by double cleavages. Plasmids carrying cloned inserts of pRK100 were additionally cleaved with AccI, BamHI, ClaI, Dral, PstI and PvuII. Restriction endonuclease digestions were carried out according to the instructions of the supplier. DNA fragments were purified from agarose gels using the GeneClean II system (Bio101).

Agarose gel electrophoresis and restriction fragment size determination. Restriction enzyme digests of pRK100 were separated by constant field (CFGE) and pulsed-field (PFGE) gel electrophoresis. For separation and size determination of restriction fragments ranging from 0.2 to 7 kb, CFGE with 0.5–2% submarine agarose gels was used. Larger fragments were separated by PFGE (Pharmacia Biotech, Gene Navigator System).

DNA hybridization for pRK100-encoded determinants. DNA labelling and hybridization experiments were carried out using the DIG DNA labelling and detection kit (Boehringer). Hybridization experiments were performed to detect the aerobactin-uptake system, ColV, the tra operon, replicons first to determine which replication regions were present and subsequently their position (RepFIB, RepFIC, Rep9) – IS1 sequences and chromosomai P, type I and S-fimbriae. The following labelled probes were used. For the aerobactin-uptake system the 2.7 kb SalI–BamHI fragment of pABN1 (De Lorenzo et al., 1986) with genes iucA and iucB was used, and for ColV the 0.5 kb PvuII–BglII fragment of pHK11 with cwaC and the overlapping cwi genes (Gison et al., 1987). All EcoRI restriction fragments of pRK100 were labelled and used to probe the Couturier bank of rep plasmids (Couturier et al., 1988): for RepFIB the 1.2 kb PstI fragment of pUBL2404; for RepFIC the 0.9 kb EcoRI–HindIII fragment of pUBL2440; for Rep9 the 0.5 kb PstI fragment of pUBL2422 (Couturier et al., 1988). For the tra operon, EcoRI fragments of pED100 and the 8.3 kb EcoRI restriction fragment f6 of pED100 (Willets & Johnson, 1981) were used, and for detection of IS1 the 2.9 kb PvuII fragment of pTC72#24 (kindly provided by M. Chandler).

The 0.9 kb EcoRI–HindIII fragment of pFDH1 and the three longest (27, 20 and 17 kb) EcoRI fragments of pRK100 were also individually labelled and used to probe EcoRI, HindIII, SalI, XhoI and double digestions of pRK100.

Stringent conditions were used for all hybridization experiments described in this report.

Colicin production. ColV and Colla production was determined using the overlay method (Pugsley, 1985) with indicator strains of the Pugsley collection of colicinogenic strains and with strains KH1038 (sensitive only to ColV), KH1044 (resistant to ColV) and AB1133 (sensitive to all colicins).

Resistance to the bactericidal action of serum. E. coli C600 and C600(pRK100), as well as E. coli K5333 with and without pRK100, were tested for serum survival or resistance. Serum survival was tested in the presence of 1% K. H1044 (resistant to ColV) and AB1133 (sensitive to all colicins).

Plasmid stability and curing. Plasmid stability was studied by inoculation of the strain into LB medium without antibiotics and incubating at 37 °C with shaking. The next day the cell suspension was diluted into fresh LB medium and again incubated at 37 °C. After 21 passages the cell suspension was diluted and plated. Colonies were then transferred to grids and tested for antibiotic resistances and colicin production.

Plasmid stability was further tested by introducing pED100, a conjugative derivative of plasmid F also of the IncF1 incompatibility group, into K5333.
## Table 1. E. coli strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant properties</th>
<th>Source or reference**</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS533</td>
<td>Rough, K1::H7 harbouring pRK100</td>
<td>Žgur-Bertok et al. (1990)</td>
</tr>
<tr>
<td>HB101</td>
<td>hsdR hsdM recA13 supE44 lacB6 lacZ proA2</td>
<td>D. Ehrlich1</td>
</tr>
<tr>
<td>DH5α</td>
<td>thi-1 recA hsdR17 lac</td>
<td>A. Francky2</td>
</tr>
<tr>
<td>C600</td>
<td>thi-1 thr-1 lacB6 lacY1 SmR</td>
<td>B. Bachmann3</td>
</tr>
<tr>
<td>AB1133</td>
<td>Sensitive to all colicins</td>
<td>B. Bachmann</td>
</tr>
<tr>
<td>KH1038</td>
<td>Sensitive only to ColV</td>
<td>K. G. Hardy4</td>
</tr>
<tr>
<td>KH1044</td>
<td>Resistant to ColV</td>
<td>K. G. Hardy</td>
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### Plasmids

<table>
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<th>Strain or plasmid</th>
<th>Relevant properties</th>
<th>Source or reference**</th>
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<td>pRK100</td>
<td>ColV plasmid with Tn5431</td>
<td>Žgur-Bertok et al. (1990)</td>
</tr>
<tr>
<td>RSF2001</td>
<td>F plasmid with KnR</td>
<td>Heffron et al. (1977)</td>
</tr>
<tr>
<td>pUC19</td>
<td>ApR</td>
<td>Yanisch-Perron et al. (1985)</td>
</tr>
<tr>
<td>pAlter</td>
<td>TcR</td>
<td>Promega</td>
</tr>
<tr>
<td>pTC72#24</td>
<td>IS1</td>
<td>M. Chandler4</td>
</tr>
<tr>
<td>pABN1</td>
<td>Aerobactin-uptake system</td>
<td>De Lorenzo et al. (1986)</td>
</tr>
<tr>
<td>pHK11</td>
<td>Colicin V</td>
<td>Gilson et al. (1987)</td>
</tr>
<tr>
<td>pED100</td>
<td>tra operon</td>
<td>Willets &amp; Johnson (1981)</td>
</tr>
<tr>
<td>pPKL4</td>
<td>Fimbriae type I</td>
<td>Ott et al. (1991)</td>
</tr>
<tr>
<td>pRHU845</td>
<td>pap/prs</td>
<td>Ott et al. (1991)</td>
</tr>
<tr>
<td>pANN801-13</td>
<td>sfa/foc</td>
<td>Ott et al. (1991)</td>
</tr>
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<td>pUBK2404</td>
<td>RepFIB</td>
<td>Couturier et al. (1988)</td>
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<tr>
<td>pUBL2440</td>
<td>RepFIC</td>
<td>Couturier et al. (1988)</td>
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<tr>
<td>pUBL2422</td>
<td>Rep9</td>
<td>Couturier et al. (1988)</td>
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<td>pFDH1</td>
<td>pAlter with the 20 kb HindIII fragment of pRK100</td>
<td>This study</td>
</tr>
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<td>pFDH2</td>
<td>pAlter with the 20 and 12 kb HindIII fragments of pRK100</td>
<td>This study</td>
</tr>
<tr>
<td>pFD5</td>
<td>pAlter with the 17-3 kb SfiI fragment of pRK100</td>
<td>This study</td>
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<td>pUX1</td>
<td>pUC19 with 0.9 kb EcoRI/HindIII of pFDH1</td>
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<td>pUV5</td>
<td>pUC19 with the 5 kb EcoRI fragment of pFDH2</td>
<td>This study</td>
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<td>pUX5</td>
<td>pUC19 with the 3.6 kb EcoRI fragment of pFDH1</td>
<td>This study</td>
</tr>
<tr>
<td>pUX600</td>
<td>pUC19 with 0.6 kb PstI fragment of pUX5</td>
<td>This study</td>
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</tbody>
</table>

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Plasmid curing was also performed with acridine orange and SDS treatment (Hardy, 1987).

**Conjugational transfer experiments.** For conjugational transfer of plasmids the donor and recipient strains were over-streaked on an LB plate and incubated overnight at 37 °C. Following the mating period, a portion of the mating mixture was removed from the growth surface with a sterile rod and was streaked on a selection plate. For liquid mating, overnight cultures of donor and recipient strains were diluted 50-fold in LB liquid medium. The donor strain was incubated at 37 °C for 3 h without shaking and the recipient strain for 2 h with shaking. A mating mixture consisting of 0.5 ml of the donor and 4.5 ml of the recipient cultures was prepared and incubated for 20 h at 37 °C. Transconjugants were selected on Simmons citrate agar plates, supplemented with Tc and Ap when testing for transfer of pRK100 and with Kn when testing for transfer of plasmid RSF2001. E. coli transconjugants were selected on LB media supplemented with Sm, Ap and Tc for transfer of pRK100 or Sm and Kn for transfer of RSF2001. Negative controls were prepared by plating donor and recipient strains separately on selective media. Transconjugants were screened for plasmid DNA.

**Production of type I fimbriae, P fimbriae, S fimbriae, CNF1 and haemolysin.** Cleaved KS533 chromosomal DNA was probed for type I fimbriae with the labelled 6 kb PstI fragment of pPKL4, for pap/prs with the 4 kb HindIII–EcoRI fragment of pRHU845 and for sfa/foc with the 6 kb ClaI–EcoRV fragment of pANN801-13. However, for testing for transfer of pRK100 or Sm and Kn for transfer of RSF2001. This study

**DNA sequencing.** Single- and double-stranded sequencing of cloned pRK100 restriction fragments was performed using the
Sequenase version 2.0 Sequencing Kit (USB) and the Silver sequencing kit (Promega). Primers were the commercially available M13/pUC forward and reverse sequencing primers.

**RESULTS**

**Location of Tn5431 and flanking regions**

Previously, we determined that pRK100 carries transposon Tn5431, encoding resistances to Tc and Ap (Zgur-Bertok et al., 1994). To determine the position of Tn5431 on pRK100, three clones carrying the antibiotic resistances of Tn5431, pFDH1, pFDH2 and pFDS1, were isolated (Fig. 1). pFDH1 harbours the 20 kb HindIII fragment, pFDH2 two consecutive HindIII fragments, 20 kb and 12 kb long (isolated by cloning partially HindIII-digested pRK100 DNA), while pFDS1 carries the 17.5 kb Sall fragment. Using restriction mapping, hybridization experiments, and testing with indicator strains for colicin activity it was determined that pFDH1 and pFDS1 harbour an incomplete Tn5431 and that each plasmid carries one flanking region. pFDH1 carries Colla, pFDS1 carries ColV, and pFDH2 carries the entire Tn5431, ColV and Colla.

To confirm the hybridization and colicin typing results, plasmids pUV55 and pUX5, with 5 kb and 3.6 kb EcoRI fragments of pFDH2, respectively, were isolated (Figs 1 and 2). Subsequently, the 0.6 kb PstI restriction fragment of pUX5 was subcloned to generate pUX600. Single-stranded sequencing of the pUV55, insert with the reverse sequencing primer and of the pUX5 insert with the forward sequencing primer showed that Tn5431 was inserted into Colla at nucleotide position 3424 (determined by alignment with the sequence deposited under EMBL accession number M13819) of the conserved ORF2, which is not involved in Colla production. Upstream of the IR-L of Tn5431 are sequences from nucleotide positions 3425 to 3727 of the 3727 bp deposited sequence. Downstream of the Tn5431 IR-RII are the colicin Ia immunity and structural genes. Single-stranded sequencing of the 0.6 kb PstI insert of pUX600 with the forward and reverse sequencing primers demonstrated conservation of sequences of the Colla structural gene, while the ORFI sequences are not conserved. Alignment of the nucleotide sequence obtained by single-stranded sequencing of pUV55 DNA with the forward primer and the ColV sequence deposited under EMBL accession number X57525 showed conservation of nucleotide sequences of the colicin V cvaC structural gene and the cvi immunity gene. Nucleotide sequencing also showed characteristic 5 bp repeats of target DNA at both Tn5431 ends, confirming that transposition had occurred.

Altogether these results demonstrated that Tn5431 had transposed into a region encoding Colla and ColV. Tn5431 is flanked on one side by ColV and on the other by Colla.

**Replication regions, aerobactin system and IS1**

Waters & Crosa (1986) reported conservation of the replication region RepFIB among all ColV plasmids investigated and, further, that most encode the aerobactin iron-uptake system with an upstream and downstream insertion sequence IS1. To detect and map these nucleotide sequences we performed hybridizations of specific labelled probes with restriction enzyme digests of pRK100 and, for comparison, with fragments of the prototypic plasmid pColV-K30.

Comparison of the hybridization signals demonstrated
ColV plasmid pRK100

conservation in pRK100 of the replicon RepFIB, a basic replicon found in large plasmids of the IncFI group (Gibbs et al., 1993) the aerobactin-uptake system and the two IS1 sequences (Figs 3, 4 and 5, respectively). Some but not all ColV plasmids also have the RepFIA homologous replicon downstream of the aerobactin-uptake system. Since large plasmids are known to carry more than one replicon, first all EcoRI fragments of pRK100 were labelled and used to probe the Couturier bank of rep plasmids. The probe did not hybridize with the 917 bp EcoRI fragment of plasmid pUBL2154 harbouring RepFIA, demonstrating that pRK100 does not have this replicon. On the other hand the probe hybridized with fragments corresponding to RepFIC (the 967 bp EcoRI–HindIII fragment of pUBL2440), Rep9 (the 539 bp PstI fragment of pULB2422), RepFIA copA (the 543 bp PstI fragment of pUBL2401) and

RepFIA copB (the 540 bp PstI fragment of pULB2402). All four are known to cross-hybridize. To determine their positions, the labelled RepFIC and Rep9 fragments were then used to probe restriction enzyme digests of pRK100. Both probes hybridized to the same two EcoRI, SalI and HindIII fragments, indicating that a replicon(s) of this family is present at two positions of pRK100 (Fig. 6).

tra region and construction of the pRK100 map

The pRK100 tra region was determined by hybridization of the labelled pED100 EcoRI fragments harbouring the tra operon of plasmid F and the labelled 8.3 kb EcoRI
fragment f6, to restriction fragments of pRK100 and pColVK30 (Figs 7 and 8, respectively). Comparison of the hybridization results for the tra region with the results obtained with the labelled 0·9 kb EcoRI-HindIII fragment of pFDH1 enabled us to deduce the position of the tra operon with regard to ColIa. Further comparison of the above results with the RepFIC and Rep9 hybridization patterns enabled us to deduce that a RepFIC replicon is downstream of ColIa. This was confirmed by single-stranded nucleotide sequencing with the forward sequencing primer of the pUX1 insert. Subsequently, by analysing the hybridization results obtained with probes specific for the aerobactin system, IS1 sequences, RepFIC and with the labelled 0·9 kb EcoRI-HindIII pFDH fragment, we were able to deduce the position of the tra operon on pRK100.

XhoI cleaves pRK100 at only two sites, resulting in two fragments, of 26 kb and 120 kb. One site was mapped in the ColIV region, while the other was deduced from the fact that neither Tn5431 nor pFDH1 has a XhoI site and from fragment patterns of double restrictions.

On the basis of the above-mentioned results together with the fragment patterns of double restrictions, hybridization experiments using the three longest individually labelled EcoRI fragments (27, 20 and 17 kb), and comparison of probe binding to pColVK30 we were able to complete the restriction map (Fig. 9).

### Increased serum survival conferred by pRK100

ColIV plasmids have been shown to enhance serum resistance. TraT, the surface exclusion protein of the plasmid transfer system, and the iss (increased serum survival) locus, which is linked to the colicin V genes, have been implicated (Binns et al., 1979). Serum survival was tested for the original clinical strain KS533, strain C600 and C600 harbouring pRK100 in the presence of 1%, 2% and 3% human serum. The results (Table 2)
Table 3. Plasmid transfer frequency to different species

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>E. coli</th>
<th>S. typhimurium</th>
<th>K. pneumoniae</th>
<th>E. cloacae</th>
<th>P. aeruginosa</th>
</tr>
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<tbody>
<tr>
<td>pRK100</td>
<td>$2 \times 10^{-2}$</td>
<td>-</td>
<td>$1 \times 10^{-3}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RSF2001*</td>
<td>$1 \times 10^{-2}$</td>
<td>$3 \times 10^{-3}$</td>
<td>$3 \times 10^{-7}$</td>
<td>-</td>
<td>NT</td>
</tr>
</tbody>
</table>

*RSF2001 is plasmid F with KnR.

show increased survival of C600 harbouring pRK100 in the presence of 1% and 2% serum (five- and fourfold, respectively) while both strains were killed in the presence of 3% serum. In contrast, both the clinical strain KS533 with plasmid pRK100 and strain KS533 without the plasmid exhibited growth at all serum concentrations tested. The K1 capsule thus offers much greater protection than any plasmid-encoded determinant.

Stability and conjugal transfer to other Gram-negative bacteria

Following 21 passages of pRK100 without antibiotic selection 1200 colonies were transferred to grids (LB plates) and tested for antibiotic resistances and colicinogenicity. All colonies retained the characteristics of the initial strain.

Stability of pRK100 was also studied by introduction of an incompatible plasmid, pED100, a CmR derivative of F. A total of 152 colonies expressing the characteristics of both plasmids, resistances to Ap, Tc, Cm and colicinogenicity, were subsequently further subcultured by replica plating first onto an LB plate and then to an LB plate supplemented with Cm. This procedure was repeated seven times, during which some colonies exhibited gradual loss of the CmR character. None of the CmR colonies exhibited loss of pRK100. Gel electrophoresis of the isolated plasmid DNA of strains which stably maintained colicinogenicity and antibiotic resistances of both plasmids (Ap, Tc, Cm) showed the presence of both plasmids or only F plasmid DNA, indicating integration of pRK100 into the chromosome. Only culturing strain KS533 in the presence of SDS yielded two colonies cured of plasmid pRK100.

To determine whether pRK100 can be transferred and maintained in other Gram-negative bacteria, conjugal transfer was attempted to S. typhimurium, K. pneumoniae, P. aeruginosa and Ent. cloacae. With plate mating only a small number of K. pneumoniae transconjugants harbouring pRK100 were isolated. Liquid matings were also carried out and the transfer frequencies of pRK100 and RSF2001 were compared (Table 3). pRK100 and RSF2001 were both transferred at low frequency to K. pneumoniae. On the other hand while the transfer frequency of RSF2001 to S. typhimurium was comparable with that to E. coli, transfer of pRK100 to S. typhimurium was never detected. Transfer of RSF2001 to Ent. cloacae was detected only with plate mating.

Chromosomal virulence-associated genes of strain KS533

Hybridization experiments demonstrated the presence of nucleotide sequences specific for type I, P and S fimbriae. PCR was carried out with primers specific for CNF1 but amplification of the corresponding fragment was not detected. KS533 does not produce haemolysin as determined by growth on blood agar plates.

DISCUSSION

Using hybridization and nucleotide sequencing a map of plasmid pRK100 and its characteristics was constructed. Transposon Tn5431 has transposed into the direct vicinity of the Colla immunity and structural genes. Prior to transposition the Colla and Colla genes were thus linked. The Colla operon has so far been determined only on plasmids of the IncF1 incompatibility group, which share replicon RepFIB, colicin V genes and – in most of the plasmids examined – the aerobactin-uptake system. On the other hand, examination of Colla-harbouring plasmids of the E. coli Reference Collection (ECOR) showed that Colla is present on large plasmids with little homology. It was therefore suggested that the Colla operon or some larger fragment has been transferred between distinct plasmid lineages (Riley & Gordon, 1992). On pRK100 the conserved Colla sequences are on one side separated from CollaV by approximately 2.6 kb and on the other side, upstream of the conserved RepFIC sequences, by approximately 1.5 kb. Together with the Colla sequences this is roughly the size of the fragment homologous to different Colla-carrying plasmids (Riley & Gordon, 1992). Sequences involved in Colla transfer could be present in the vicinity of the Colla and CollV determinants of pRK100.

Using specific probes we demonstrated that pRK100 harbours RepFIB and RepFIC. It is not yet known
Fig. 10. Comparison of the map of pRK100 with those of plasmid F (adapted from Neville et al., 1996) and the ColV plasmids pColV-K30 and pColV-B188 (Waters et al., 1989). The location of insertion sequences (IS) is shown by black boxes. The IS1 copy which is believed to be partial is labelled in parentheses. Dotted lines indicate that the location of the tra region is approximate, within the limits of available restriction enzyme sites. Numbers within the maps indicate kilobase coordinates.

whether pRK100 carries a complete and functional RepFIC replicon. However, when pRK100 was challenged with an incompatible plasmid, integration of pRK100 into the chromosome or the coexistence of both plasmids was observed, indicating that pRK100 has two functional replication regions. Further, on the basis of binding of probes specific for RepFIC and the tra region we demonstrated that in pRK100 the tra region is, as in plasmid F, linked to the RepFIC replication region. Other ColV plasmids, including pColV-K30, also harbour RepFIC sequences; however, their locations have not been determined (Bergquist et al., 1986).

Colicinogenicity and conjugal transfer contribute to plasmid stability in bacterial populations. Cells which have lost the plasmid are no longer immune and can be killed by plasmid-harbouring cells. Further, cells which have lost the plasmid can act as recipients in conjugal transfer. However, our experiments demonstrated pronounced in vitro stability of pRK100. Colonies cured of the plasmid were isolated only by SDS treatment. Further, the spontaneous loss of pRK100 upon subculturing and storage was never observed. Introduction of an incompatible plasmid could result in integration of pRK100 into the chromosome, probably by recombination between chromosomal and plasmid insertion sequences. Integration of plasmids is known to reduce the expression of plasmid-encoded factors due to differential supercoiling of the integrated plasmid DNA (Ott, 1993). It might also be viewed as a means by which selfish DNA avoids elimination in conditions detrimental to maintenance. The influence of environmental signals in the regulation of integration will be investigated. On plasmids such as pRK100, the maintenance of genes is stable while having, with regard to chromosomal genes, the additional advantage of being able to disseminate through a population by conjugation.

As well as plasmid-encoded determinants, strain KS533 also has chromosomal virulence determinants important for eliciting extraintestinal infections. The K1 capsule is poorly immunogenic and is responsible for immunotolerance by the host. Type F fimbriae mediate the initial binding of uropathogenic E. coli to its host receptor. Fifteen per cent of the ECOR strains possess F-related plasmids (Boyd et al., 1996). The Salmonella Reference Collection A (SARA) also has approximately the same percentage of F-like plasmids and it has been inferred that F plasmid transfer is an important mechanism of interspecies recombination (Boyd & Hartl, 1997). Salmonella IncF1 R plasmids are known to carry the ISI-bound aerobactin-uptake system (Colonna et al., 1985) and some Salmonella isolates harbour colicin plasmids (Ayala et al., 1994). Besides, some E. coli virulence factors might have originated in some other species (Mühl dorfer & Hacker, 1994). On the other hand, ColV plasmids have been determined only in E. coli even though the replication and transfer regions of plasmid F and ColV plasmids are homologous and exhibit a high degree of sequence conservation (Gibbs et al., 1993). It could be that for some reason DNA restriction and modification is more efficient in reducing or eliminating recombinants carrying ColV plasmid than F plasmid recombinants. In our plasmid-transfer experiments only K. pneumoniae transconjugants harbouring pRK100 were isolated. By comparing the host range of plasmids RSF2001 and pRK100 we see that the latter cannot be transferred to S. typhimurium or Ent. cloacae even though both plasmids are transferred to another E. coli.
strain with approximately the same frequencies. The absence of the RepFIA replication region in pRK100 could also be responsible for the plasmid's limited host range when compared with plasmid F. Plasmid host range is important particularly for transferable plasmids. Conjugal transfer of plasmid-encoded virulence properties to another strain or species could provide quantum leaps toward virulence. The role of RepFIA in plasmid host range will be investigated. As environmental stimuli regulate gene expression and gene transfer (Mel & Mekalanos, 1996), experiments will be carried out to determine the in vivo stability and transfer of pRK100.

The maps of plasmids pRK100, F and two other known ColV plasmids – pColV-K30 and pColV-B188 – are presented in Fig. 10. The tra regions of the ColV plasmids are approximate, but with regard to the well-studied tra region of plasmid F should span about 30 kb. The aerobactin iron-uptake system which is encoded by the aerobactin system integrated via IS1-mediated recombination could have been important in the evolution of ColV plasmids. It can be envisaged that the aerobactin system integrated via IS1-mediated recombination into the region between the RepFIA and RepFIA replicons of an ancestral F-like plasmid. Re-</(0,0,999,999)matplotlib.figure.Figure object at 0x7f752bca05f0>-combination between two copies of IS1 could result in deletion of the RepFIA replication region.

It is evident that pRK100 evolved in several steps. First a segment encoding the ColIa operon was transferred from one plasmid to a ColV plasmid, then transposon Tn5431 transposed into sequences in the direct vicinity of the ColIa genes, the role of certain replicons in plasmid host range and gene clustering on large plasmids encoding virulence properties.

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