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Physical and Genetic Characterization of the IncI Plasmid R144-*drd*3[1]

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A physical and genetic map of the IncI plasmid R144-*drd*3 was obtained by determining restriction endonuclease sites and by physical and genetic analysis of cloned fragments, of Tn*1* insertion mutants and of deletion mutants.

Plasmid R144 (*1*) is a self-transmissible plasmid which belongs to incompatibility group I (IncI) (*2*). R144 determines several well-known properties: the synthesis of colicin Ib (Col), immunity to colicin Ib (Imm), resistance for kanamycin (Km^R), (entry) exclusion (Exc) (*3*), and suppression of *dnaG* mutations in *Escherichia coli* K-12 (Sog) (*4*). A physical and genetic map of R144 might be helpful to understand the relationship with the closely related IncI plasmid ColIb for which such a map was presented recently (*5,6*). The R144 plasmids mentioned in this paper have derepressed transfer genes, i.e., R144 and R144::Tn*10* are actually R144-*drd*3 and R144::Tn*10*-*drd*3.

PHYSICAL CHARACTERIZATION

The physical map of R144 is presented in Fig. 1. To map *Hin*dIII, *Bgl*II, *Bam*HI, *Eco*RI, and *Sal*I cleavage sites, single and double digestions of R144-DNA with these restriction enzymes were performed and the digestion products were subsequently analysed by electrophoresis on 0.6% agarose gels. Plasmid R144 appeared not to be cleaved by *Bam*HI. Based on the molecular weights of the fragments generated in the single and double digestions, R144 has a molecular mass of 67×10^6 Da (67 MDa). Additional data required for the physical map of R144 were obtained by isolating the *Hin*dIII fragments H1 till H9 (see the legend of Fig. 1) and the *Bgl*II fragments B1 till B6 from agarose gels (*7*), followed by digestion of these fragments with respectively *Bgl*II and *Hin*dIII. These secondary digestion products were analyzed by agarose gel electrophoresis. Besides, *Hin*dIII and *Bgl*II generated fragments of R144 were cloned in the *Hin*dIII cleavage site of pACYC184 (*8*) and the *Bam*HI cleavage site of pBR322 (*9*). The resulting recombinant plasmids were characterized by digestion with appropriate restriction endonucleases.

The following fragments were cloned: H1 (in pRAH201); H3 (in pRAH202); H4 + H5 (in pRAH203); B2 (in pRAH310) and B9 (in pRAH312). Finally, miniplasmids obtained after digestion of R144-DNA with *Bgl*II followed by ligation were analyzed. Two miniplasmids were used: pR144-1 consisting of fragment B2 and pR144-2 originating from a partial digestion product in the mixture and consisting of fragments B2 and B5.

GENETIC CHARACTERIZATIONS

Miniplasmid pR144-1 expressed R144 genes for autonomous replication (*rep*) as well as the genes coding for Km^R, for colicin Ib production (*col*), and colicin immunity (*imm*). The presence of the *col* gene on fragment B2 is consistent with the observation that colicin Ib production is specified by an

[1] More complete documentation is on file at the editorial office of *Plasmid*.

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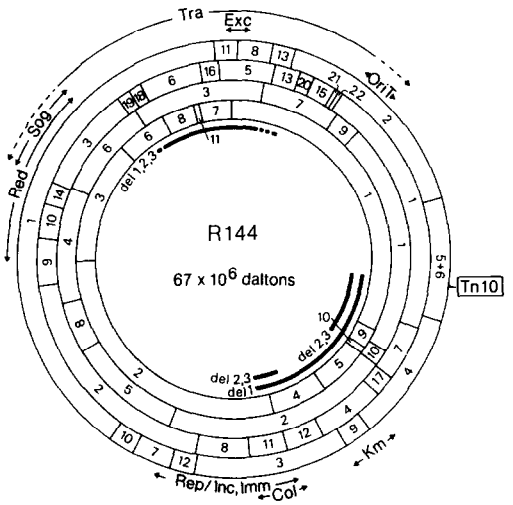

FIG. 1. The physical and genetic map of R144. The inner circle represents the *Hin*dIII cleavage map; the second circle, the *Bgl*II map; the third circle, the *Eco*RI map and the outer circle, the *Sal*I map. The numbers in the figure correspond with the numbers used in the text. H1 till H11 are fragments with molecular masses of, respectively, 22.0, 19.5, 8.5, 3.80, 3.40, 3.30, 2.10, 2.10, 1.40, 0.38, and 0.34 MDa. A further *Hin*dIII fragment of 0.10 MDa was too small to be positioned and is not indicated in the figure. B1 till B10 are *Bgl*II fragments (14.5, 14.0, 8.0, 8.0, 6.0, 5.8, 4.7, 3.10, 1.65, and 1.15 MDa). E1 till E22 are *Eco*RI fragments (15.1, 13.5, 5.5, 3.60, 3.50, 3.20, 3.20, 2.95, 2.80, 2.00, 1.95, 1.95, 1.40, 1.30, 1.30, 0.99, 0.99, 0.75, 0.68, 0.68, 0.20, and 0.20). S1 till S13 are *Sal*I fragments (25.0, 11.0, 8.3, 6.8, 3.35, 2.68, 1.88, 1.78, 1.45, 1.32, 1.32, 1.20, and 1.00). The relative positions of S5 and S6 are not determined. Tn*10* indicates the approximate position of the transposon in plasmid R144::Tn*10*. The lines designated del1, del2, and del3 indicate the R144-deleted region in the deletion derivatives of R144::Tn*10* (numbers 1, 2, and 3). The approximate positions of the regions determining Tra, Exc, Sog, Rep/Inc, Col, Imm, Km^R, Red, or carrying *oriT* are indicated by the double arrows.

*Eco*RI fragment (probably E11) of about 2 MDa (R. A. Hartskeerl, unpublished results). pRAH203 expressed Km^R but did not specify colicin Ib synthesis nor colicin immunity. For the expression of Km^R, fragments H4 and H5 must be in proper orientation. Apparently *Hin*dIII cleaves the Km^R gene. Cells harboring the pBR322-B2 hybrid pRAH310 appeared 20 times less resistant to ampicillin (Ap^R) than cells harboring pBR322. We assume that the copy number of pRAH310 is about 20 times lower than the copy number of pBR322, most likely as a consequence of the presence of R144 genes for stringent replication control. In matings between an R144::Tn*10*$^+$ donor and recipients harboring pRAH310, respectively, pBR322, the number of transconjugants receiving the donor plasmid was about equal. However, when the transconjugants were cultivated under non-selective conditions R144::Tn*10* and pRAH310 appeared incompatible; apparently they share common replication regulators or replication sites. R144 and the closely related plasmid ColIb are able to suppress *dnaG* mutations in *E. coli* K-12, they carry the *sog* gene. In ColIb a 5.3-MDa-size *Eco*RI fragment was found to contain the *sog* gene (*4,10*). R144 contains an *Eco*RI fragment of about the same size (fragment E3, Fig. 1). Fragment E3 of R144 can be cleaved by *Hin*dIII. The main part of fragment E3 is thus present in the *Hin*dIII fragment H3 (Fig. 1). pRAH202, carrying fragment H3, was able to suppress the *dnaG* mutation in strain AB4004 (*11*) thus H3, probably the part that overlaps E3, harbors the *sog* gene.

The pBR322 derived hybrid pRAH312 is effectively transmitted by R144 from a *recA* background and appeared to be present in the transconjugants as an independent molecular species. This transfer of pRAH312 is apparently based on mobilization. Since the vector pBR322 itself is transmitted by R144 at a very low level, the fragment B9 in this plasmid harbors the transfer origin of R144 (*oriT*). The *exc* gene(s) (*3*), are located on fragment B3. The gene(s) are cleaved by *Sal*I (cleavage site between the fragments S8 and S11). Considering the physical map (Fig. 1) the *exc* gene(s) could be located on H1. However, pRAH201 (with fragment H1) did not demonstrate exclusion toward R144. The region determining or controlling R144 specific exclusion is apparently cleaved by *Hin*dIII. A region involved in the self-transmissibility (Tra) of R144 was identified after Tn*1* mutagenesis and deletion mutagenesis. After Tn*1* mutagenesis we selected four mutants which appeared Tra^- since they could

not transfer Km^R nor Ap^R in crosses. The Tn*1* insertions were located in the fragments H6, B6, or B7. Deletions in R144 were created by packaging the plasmid in phage P1. With R144 itself we did not find any deletions, however, the technique appeared successful with R144::Tn*10* (*3*). Among transductants obtained with P1 propagated on a host carrying R144::Tn*10*, selected either for tetracycline resistance (Tc^R) or for Km^R we found a number of deleted plasmids. Two Tc^R transductants were phenotypically Km^S, Col^-, and Tra^- and 24 Km^R colonies had the phenotype Tc^S, Col^-, Tra^-. Analysis of the plasmids of, respectively, one Tc^R and two Km^R transductants revealed surprisingly that two or more plasmid regions were deleted (Fig. 1). Consideration of the insertion mutants, together with the deletion mutants, suggests a possibly continuous Tra region in R144. Since conjugal transfer requires *oriT* we included the fragment B9 in the Tra region. Wilkins *et al.* (*4*) reported that the *sog* gene might be a transfer gene, therefore we also included the fragment E3 in the Tra region. Based on these assumptions, a continuous Tra region of about 20 MDa is indicated, however, we cannot exclude that the transfer genes are clustered in more than one region, as has been reported for RP4 (*12*). It is noteworthy that the number of transconjugant colonies in matings between an $R144^+$ donor and a recipient harboring pRAH202 was about six times lower than in matings between the same $R144^+$ donor and a recipient harboring pACYC184. The gene(s) coding for the function(s) involved in this conjugation reduction, designated Red in Fig. 1, could be part of the Tra region of R144 and hence the expression of the gene(s) could be controlled by the *drd* gene. This mating reduction might be the cause for the strongly reduced efficiency of matings between an R144::Tn*10*$^+$ donor and a recipient harboring R144-*drd*3 compared with the matings done with a recipient harboring R144-*drd*$^+$ (manuscript in preparation). A detailed comparison between the maps of R144 and ColIb is beyond the scope of this report. It is clear, however, that besides similarities there are also significant differences.

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