

## The *EcoDXX1* Restriction and Modification System: Cloning the Genes and Homology to Type I Restriction and Modification Systems

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Received July 18, 1988; revised December 28, 1988

The *Escherichia coli* plasmid pDXX1 codes for a type I restriction and modification system, *EcoDXX1*. A 15.5-kb *Bam*HI fragment from pDXX1 has been cloned and contains the *hsdR*, *hsdM*, and *hsdS* genes that encode the *EcoDXX1* system. The *EcoDXX1 hsd* genes can complement the gene products of the *EcoR124* and *EcoR124/3 hsd* systems, but not those of *EcoK* and *EcoB*. Hybridization experiments using *EcoDXX1 hsd* genes as a probe demonstrate homology between *EcoDXX1* and *EcoR124* and *EcoR124/3* restriction-modification systems, but weak or no homology between *EcoDXX1* and *EcoK* or *EcoB* systems. © 1989 Academic Press, Inc.

The type I restriction and modification (R-M)<sup>1</sup> systems of Enterobacteriaceae are the best-characterized R-M systems (for recent review see Yuan, 1981; Bickle, 1982). Genetic analysis of *Escherichia coli* and *Salmonella* type I R-M systems has shown that they are coded by three closely linked chromosomal genes (Boyer and Roulland-Dussoix, 1969; Hubacek and Glover, 1970). Biochemical, genetic, and immunological evidence has clearly shown that chromosomally encoded type I R-M systems of *E. coli* and *Salmonella* can be divided, as proposed by Price *et al.* (1987), into two genetically and antigenically distinct families. The first group (IA) is represented by *EcoK*, *EcoB*, *EcoD*, *StySB*, *StySP*, and *StySQ*, and the second group (IB) by *EcoA* and *EcoE*.

Recent findings that R-M systems encoded by *E. coli* plasmids have typical type I biochemical properties and genetic organization with respect to the *hsd* genes (Piekarowicz *et al.*, 1985; Piekarowicz and Goguen, 1986; Firman *et al.*, 1985; Price *et al.*, 1987) raise the question of homology between them, as well as to those chromosomally encoded R-M systems of *E. coli* and *Salmonella*. Recently, it was shown that the two *E. coli* plasmid-encoded type I R-M systems, *EcoR124* and

*EcoR124/3*, are related to each other but are distinct from the two previously studied type I restriction enzymes (Price *et al.*, 1987).

In this paper, we report the molecular cloning, genetic organization, and homology of the *EcoDXX1* R-M system (Piekarowicz *et al.*, 1985) to other type I systems.

### MATERIALS AND METHODS

*Bacterial strains and culture conditions.* All strains used were *E. coli* and are listed in Table 1. Media and buffers used for phage plating and bacterial manipulations were as described in Maniatis *et al.* (1982). When necessary, antibiotics were used in the following final concentrations: tetracycline, 20 µg/ml; chloramphenicol, 20 and 200 µg/ml; kanamycin, 20 µg/ml; and ampicillin, 20 µg/ml.

*Isolation of plasmid and bacteriophage DNA.* The bacteriophage and plasmids used are described in Table 1. Minipreparations of plasmid DNAs were prepared using the alkaline lysis method of Brinboim and Dolly (1979). Highly purified DNA was isolated by cesium chloride-ethidium bromide centrifugation of a cleared lysate, as described by Clewell and Helinski (1969). Unmodified and modified λ phages and their DNA were prepared on appropriate host strains as described by Maniatis *et al.* (1982).

<sup>1</sup> Abbreviations used: R-M, restriction and modification; SDS, sodium dodecyl sulfate; eop, efficiency of plating.

TABLE 1  
LIST OF BACTERIA, PLASMIDS, AND BACTERIOPHAGES

Strain	Genotype	Source or reference
<b>Bacteria</b>		
LA101	F <sup>-</sup> <i>hsdR</i> ( <i>r<sub>K</sub>m<sub>K</sub></i> ) <i>supE44</i> , <i>supF58</i> <i>lacY1</i> <i>galK2</i> , <i>galT22</i> <i>metB1</i> <i>trpR55</i>	R. Brzeziński
C(P2)	—	S. W. Glover
HB101	F <sup>-</sup> <i>hsdS20</i> <i>recA13</i> <i>ara-14</i> <i>proA2</i> <i>lacY1</i> <i>galK2</i> <i>rsoL20</i> <i>xyl-5</i> <i>mtl-1</i> <i>supE44</i> $\lambda^-$	Boyer and Roulland-Dussoix (1969)
DH1	F <sup>-</sup> <i>recA1</i> <i>endA1</i> <i>gyrA96</i> <i>thi-1</i> <i>hsdR17</i> <i>supE44</i> <i>relA1</i> $\lambda^-$	Hanahan (1983)
BHB2688	N205 <i>recA</i> [ $\lambda$ imm, 434 $\lambda$ clts <i>b2</i> <i>red</i> <i>Eam</i> <i>Sam</i> / $\lambda$ ]	Hohn (1979)
BHB2690	N205 <i>recA</i> [ $\lambda$ imm 434 $\lambda$ clts <i>b2</i> <i>red</i> <i>Dam</i> <i>Sam</i> / $\lambda$ ]	Hohn (1979)
NM430	K12 <i>lacZam</i> <i>his</i> <i>trpR</i> <i>hsdR</i>	Dept. of Genetics Warsaw University
JM107	<i>endA1</i> <i>gyr96</i> <i>thi</i> <i>hsdR17</i> <i>supE44</i> <i>relA1</i> $\lambda^-$ $\Delta$ ( <i>lac</i> , <i>proAB</i> ) [F' <i>traD36</i> <i>proAB</i> <i>lacI<sup>s</sup></i> Z $\Delta$ M15]	Yanish-Perron <i>et al.</i> (1985)
<b>Bacteriophages</b>		
$\lambda$ vir		Jacob and Weisman (1954)
$\lambda$ L47.1	( <i>srI</i> 1-2) <i>imm434cl</i> NIN5	Loenen and Brammar (1980)
$\lambda$ hsd No1		This paper
$\lambda$ ::Tn5	<i>clts</i> , <i>b221</i> , <i>Oam29</i> , <i>Pam80</i> <i>rex</i> ::Tn5	S. Iida
<b>Plasmids</b>		
pDXX1	Res <sup>+</sup> Mod <sup>+</sup> (DXX1), <i>lac</i> <sup>+</sup>	Piekarowicz <i>et al.</i> (1985)
pYA15	Res <sup>+</sup> Mod <sup>+</sup> (YA15)	Piekarowicz <i>et al.</i> (1985)
R124	Tc <sup>r</sup> , Res <sup>+</sup> Mod <sup>+</sup> (R124)	Hedges and Datta (1972)
R124/3	Tc <sup>r</sup> , Res <sup>+</sup> Mod <sup>+</sup> (R124/3)	S. W. Glover
pBR327	Amp <sup>r</sup> , Tc <sup>r</sup>	Soberon <i>et al.</i> (1980)
pBR322	Amp <sup>r</sup> , Tc <sup>r</sup>	Bolivar <i>et al.</i> (1977)
pACYC184	Amp <sup>r</sup> , Cm <sup>r</sup>	T. A. Bickle
pACYC177	Amp <sup>r</sup> , Kan <sup>r</sup>	T. A. Bickle
pUC8	Amp <sup>r</sup>	Vieira and Messing (1982)
pUNG20	Res <sup>+</sup> Mod <sup>+</sup> (R124)	Firman <i>et al.</i> (1985)
pUNG31	Res <sup>+</sup> Mod <sup>+</sup> (R124/3)	Firman <i>et al.</i> (1985)
pCP1005::Tn1000	Res <sup>-</sup> Mod <sup>+</sup> (R124)	Firman <i>et al.</i> (1985)
pUNG20-96	<i>EcoRI</i> subclone of pUNG20	This paper
pUNG20-814	<i>EcoRI-HindIII</i> subclone of pUNG20	This paper
pES7	Res <sup>+</sup> Mod <sup>+</sup> (DXX1)	This paper
PES58	Res <sup>-</sup> Mod <sup>+</sup> (DXX1)	This paper
pES109	Res <sup>+</sup> Mod <sup>+</sup> (DXX1)	This paper
pES195	Res <sup>-</sup> Mod <sup>+</sup> (DXX1)	This paper
pES14	Res <sup>+</sup> Mod <sup>+</sup> (DXX1)	This paper

**DNA manipulations.** Restriction enzymes were either purchased from Biochemicals Research Laboratories (Bethesda) or made at the Department of Genetics, Warsaw University. Reaction conditions were as described in the manufacturer's instructions. Recombinant plasmids and bacteriophage  $\lambda$  recombinants

were produced by the standard techniques described by Maniatis *et al.* (1982). *E. coli* strains were transformed by the calcium chloride method described by Maniatis *et al.* (1982). *In vitro* packaging of bacteriophage  $\lambda$  recombinant DNAs was carried out according to Maniatis *et al.* (1982). Preparation of pack-

aging extracts (Hohn, 1979) and infection of *E. coli* strains were carried out according to Protocol I described in Maniatis *et al.* (1982). Phage  $\lambda$  DNA cleaved with *EcoRI* and *HindIII* and pBR322 DNA cleaved with *HaeIII* served as molecular size standards. DNA fragments were separated on either 0.6–1.0% (w/v) agarose or 2% (w/v) polyacrylamide gels. The DNA bands were visualized by staining the gel in ethidium bromide solution (0.5  $\mu\text{g/ml}$ ) for 30 min and visualization on a shortwave uv transilluminator.

**Nick-translation and Southern hybridization.** DNA probes were nick-translated with [ $\alpha$ - $^{32}\text{P}$ ]dATP and [ $\alpha$ - $^{32}\text{P}$ ]dCTP to a specific activity of approximately  $5 \times 10^7$ – $1 \times 10^8$  dpm/ $\mu\text{g}$ , following the technique described by Rigby *et al.* (1977), and purified on a small Sephadex G-100 column. DNA fragments, separated on agarose gels, were blotted onto nitrocellulose BA85 sheets (Schleicher & Schuell) according to the procedure of Southern (1975). Prehybridization and hybridization were accomplished at 42°C in 50% (v/v) formamide, 0.1% (w/v) SDS, 0.9 M NaCl, 0.005 M EDTA, 0.05 M sodium phosphate buffer, pH 7.7. Heparin (500  $\mu\text{g/ml}$ ) was included for background control. Final washing was performed at 50°C in 0.1% (w/v) SDS, 0.018 M NaCl,  $10^{-4}$  M EDTA,  $10^{-3}$  M sodium phosphate buffer, pH 7.7.

**Transposon mutagenesis.** Mutations of the plasmid pES14 were obtained with the use of  $\lambda$ ::Tn5, as described by Berg (1977) and Berg *et al.* (1975). After infection with  $\lambda$ ::Tn5, Kan<sup>r</sup> clones were pooled and the plasmid DNAs isolated. These DNAs were used to transform *E. coli* DH1 strain. Kan<sup>r</sup>, Amp<sup>r</sup> colonies were identified and their restriction and modification phenotypes were examined. The plasmids were purified and their restriction maps were constructed and compared with the Tn5 (Rothstein *et al.*, 1980) and pES14 maps (Fig. 3).

## RESULTS

### Cloning the *EcoDXX1* R-M Genes

We have previously shown that the 40-kb pDXX1 plasmid encodes a type I R-M system

(Piekarowicz *et al.*, 1985). In order to identify where the R-M genes are located, the *BamHI* fragments were cloned into the vector  $\lambda\text{L47.1}$  and the recombinant phages containing R-M genes were selected according to the method described by Borck *et al.* (1976). This method is based on the assumption that recombinant phages containing *EcoDXX1* host-specific genes can modify their own DNA, protecting themselves against pDXX1 restriction upon transfer from the nonmodifying *E. coli* strain LA101 to the strain with the original pDXX1 plasmid. *E. coli* strain ET7 carries two plasmids, pDXX1 and pYA15, both of which code for type I restriction and modification systems, and for this reason, it could not be used for selection of the recombinants carrying *EcoDXX1 hsd* genes. On the other hand, we were able to transfer pDXX1 plasmid only to *E. coli* strain HB101. Although *E. coli* HB101 carries the *recA* gene and  $\lambda\text{L47.1}$  is able to grow on this strain with a plating efficiency of  $10^{-4}$ , compared to wild-type strains, we were forced to use the HB101 (pDXX1) strain in order to select the recombinant phages carrying *EcoDXX1 hsd* genes.

Plasmid pDXX1 DNA was digested with *BamHI* and the resulting fragments were inserted into  $\lambda\text{L47.1}$  DNA that had been circularized by ligation of its cohesive ends, and then digested with *BamHI* and *XhoI*. After *in vitro* packaging, the resulting phages were plated on strain LA101 and the progeny phages were used to infect *E. coli* strain C(P2) in order to select the recombinant phages (Loenen and Brammar, 1980). Two cycles of infection on *E. coli* strains HB101 (pDXX1) and C(P2) were employed to select the recombinants carrying *EcoDXX1 hsd* genes. Individual plaques of phage were tested for efficiency of plating (eop) on *E. coli* strains HB101 and HB101 (pDXX1) and those which formed plaques with equal eop on both strains were presumed to carry the *EcoDXX1* R-M genes. Several such phages were isolated and one,  $\lambda\text{hsd No1}$ , was used for further studies. Restriction analysis indicated that this phage contained a 15.5-kb insert that hybridized to an identical 15.5-kb *BamHI* fragment, a single

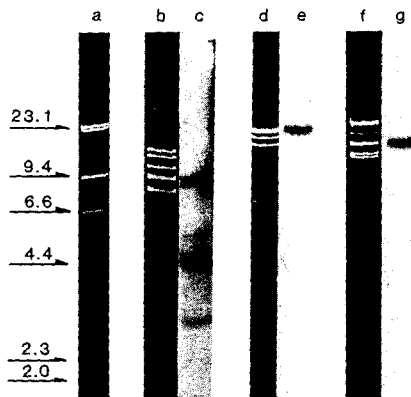


FIG. 1. Southern blotting of  $\lambda$ hsd No1 to pDXX1 DNA. The restricted DNA of pDXX1 was transferred from 1% agarose to nitrocellulose filter and probed with  $^{32}$ P-labeled  $\lambda$ hsd No1 DNA. Lanes b, d, and f show restriction endonuclease fragments and lanes c, e, and g hybridization signals from pDXX1 DNA. The restriction enzymes used were *EcoRI* (b, c), *HindIII* (d, e), and *BamHI* (f, g). Lane a shows  $\lambda$ cl857 DNA digested with *HindIII* used as a molecular weight marker (kb).

20-kb *HindIII* fragment, and several *EcoRI* fragments from pDXX1 (Fig. 1).

Since  $\lambda$ hsd No1 was not restricted *in vivo* by the *EcoDXX1* system, and DNA from  $\lambda$ hsd No1 did not serve as a substrate for the DNA-dependent ATPase or methylase activities of purified *EcoDXX1* restriction enzyme (data not presented), we concluded that this phage encodes at least the modification activity of the *EcoDXX1* system.

Attempts to subclone the *BamHI* fragment into several plasmid vectors (pBR322, pBR327, pACYC184, pACYC177) and the HB101 strain as a host were unsuccessful; however, it was possible to subclone into the *BamHI* site of pBR327 when the HB101 (pDXX1) strain was used as a host. This re-

sulted in the isolation of strains carrying both pDXX1 and the recombinant plasmids with the inserted *BamHI* fragment of pDXX1 carrying the R-M region. We were able to transfer DNA from the recombinant plasmids to strain DH1, but not to several other strains. Plasmid pES7, one recombinant plasmid obtained in this way, was used for further studies.

The level of restriction of  $\lambda$  phage shown by strains carrying the recombinant plasmid pES7 was not stable, varying from  $1 \times 10^{-5}$  to  $1 \times 10^{-2}$ , even when cultures of DH1 (pES7) were kept under the selection pressure. When single colonies from the culture that had shown the restriction level of  $1 \times 10^{-5}$  were checked, only 30 to 50% showed such a high level, while the others showed a level of about  $1 \times 10^{-2}$ . Nevertheless, this proved that the cloned *BamHI* fragment carries both the restriction and the modification genes of the *EcoDXX1* R-M system. On the other hand, strains that had both pDXX1 and recombinant plasmids showed a higher level of restriction than the strain carrying pDXX1 alone (Table 2) (Piekarowicz *et al.*, 1985). The preliminary results showed that the lower level of restriction expressed by the strain carrying plasmid pES7 was due in part to the unstable expression of the *hsd* R gene (E. Skrzypek, unpublished results).

#### Relative Positions of the *hsd* *EcoDXX1* Genes

The restriction enzyme analysis showed no differences between the *BamHI* fragment of pDXX1 originally cloned into  $\lambda$ L47.1 and the same fragment subcloned into plasmid pBR327 (Fig. 1). The localization of the R-M genes was achieved by subcloning the DNA

TABLE 2

EFFICIENCY OF PLATING OF  $\lambda$  ON THE CLONED *EcoDXX1* R-M SYSTEM

	HB101	HB 101 (pDXX1)	HB101 (pDXX1, pES7)	DH1 (pES7)	DH1 (pES109)	DH1 (pES14)
$\lambda$ .K	1	$10^{-4}$	$10^{-7}$	$10^{-2}$ - $10^{-5}$	$10^{-2}$ - $10^{-5}$	$10^{-5}$
$\lambda$ .DXX1	1	1	1	1	1	1

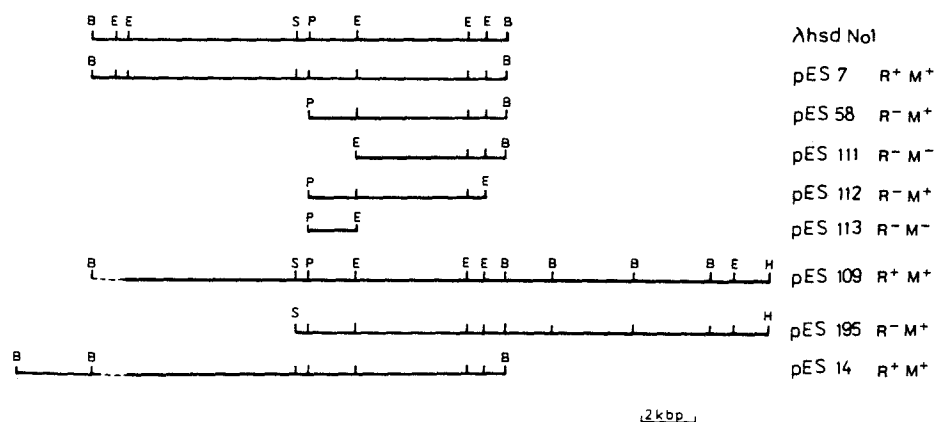


FIG. 2. Restriction fragment maps of the 15.5-kb *Bam*HI fragment of pDXX1 cloned into  $\lambda$ L47.1 vector and the subclones of *λ*hsd No1, pES7, and pES109 carrying the *EcoDXX1* *hsd* genes. Only the DNA fragments originated from pDXX1 plasmid are shown. The dotted line represents the deletion region acquired during construction of the pES109 plasmid. The phenotypes of the relevant strains are given on the right. The restriction sites are B, *Bam*HI; E, *Eco*RI; S, *Sal*I; P, *Pst*I; and H, *Hind*III.

fragments of pDXX1 DNA present in clone pES7. Plasmid pES58 was constructed by subcloning the *Pst*I-*Bam*HI fragment of pES7 into pACYC177, as well as subcloning the *Pst*I-*Eco*RI fragments of pES58 into pBR327, resulting in the isolation of the pES111, pES112, and pES113 clones (Fig. 2). None of these subclones was able to restrict  $\lambda$  phage, while pES58 and pES112 expressed modification properties specific for the *EcoDXX1* R-M system. Therefore, the modification properties of the *EcoDXX1* R-M system must be coded by the DNA sequences within a 7.0-kb *Pst*I-*Eco*RI fragment located to the right end of the 15.5-kb *Bam*HI fragment of pDXX1, and a part of a 7.0-kb *Bam*HI-*Pst*I fragment located at the left part of the cloned DNA fragment is necessary for a functional expression of the *hsdR* gene (Fig. 2). All subclones derived from pES7 could be transferred very easily to any of the *E. coli* strains. The presence of pES58 and pES112 in HB101 (pDXX1) increased the level of restriction above the level expressed by the HB101 (pDXX1) strain (unpublished data).

Because pES7 showed unstable expression of the *hsdR* gene, we wanted to know whether the fragments outside the 15.5-kb *Bam*HI fragment of pDXX1 influenced its expression. The data presented in Fig. 1 show that *Bam*HI

fragments carried by *λ*hsd No1 hybridized to 20-kb *Hind*III fragments of pDXX1. In a separate experiment, we cloned the *Hind*III fragment of pDXX1 into pBR327. The resulting plasmid, pES109, acquired a small deletion on the border between the cloned fragment and the plasmid vector DNA (Fig. 2). This deletion had no effect on the expression of the R-M genes. Plasmid pES109 expressed both restriction and modification properties but, contrary to pES7, it could be transferred very easily to several *E. coli* strains. Similar to pES7, it appeared to be restriction proficient; however, the expression of the *hsdR* gene was unstable and lower than that in the original pDXX1 plasmid. Subcloning of the *Hind*III-*Sal*I fragment of pES109 into pACYC177 resulted in the construction of plasmid pES195, which expressed only the modification properties (Fig. 2).

To obtain a stable restriction-proficient strain, we cloned the *Bam*HI fragments of pES109 into the pBR327 vector (Fig. 2). The *E. coli* DH1 cells carrying plasmid pES14 present more stable *EcoDXX1* system activity and increased levels of restriction of  $\lambda$  phage compared to other clones (Table 2). We used  $\lambda$ ::Tn5 to locate the *hsdR*, *hsdM*, and *hsdS* genes in *Bam*HI insert pES14. Potential Tn5 insertion mutants were tested and a number

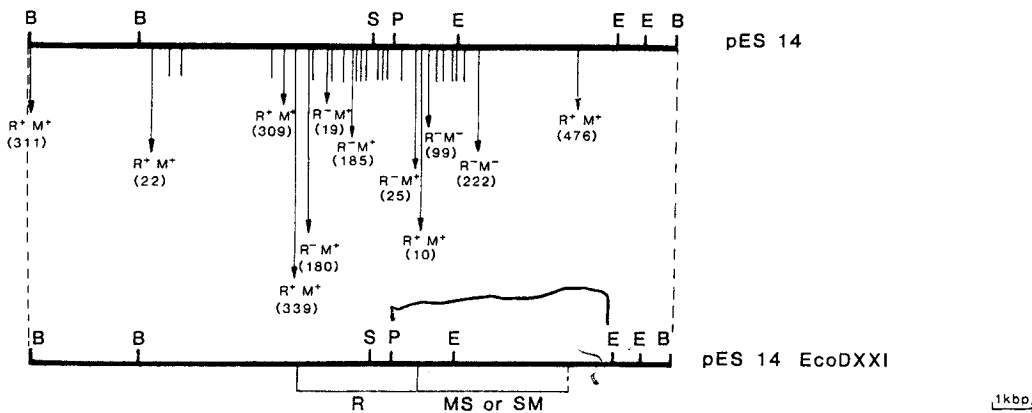


FIG. 3. Insertional mutants of the cloned *EcoDXX1* genes. Upper horizontal line shows the location of the points of insertion of Tn5 ( $\downarrow$ ) and the phenotypes of the pES14::Tn5 mutants. The bottom line shows an expanded segment of the cloned DNA and the position of the genes of the *EcoDXX1* system. The restriction sites are B, *Bam*HI; E, *Eco*R1; S, *Sal*I; and P, *Pst*I. Vertical lines with numbers show the phenotypes of different *EcoDXX1* *hsd* mutants obtained after transposon mutagenesis. Vertical lines without numbers represent mutants that have the same phenotypes as those of the flanking mutants.

of restriction- or modification-deficient mutants were isolated. Different pES14::Tn5 plasmids were analyzed by restriction enzyme digestion and the results are presented in Fig. 3.

#### Complementation between *EcoDXX1* and Other Type I R-M Systems

The approximate localization of the DNA sequences coding for restriction and modification properties allowed the use of particular

clones to examine complementation between *EcoDXX1* and other type I R-M systems. First, we tested complementation between the *EcoDXX1* *hsd* system and the *E. coli* chromosomal type I systems. Plasmid pES58 was introduced into several *E. coli* strains carrying different mutations in the *hsd* regions (data not presented). It was assumed that if the *EcoK* and *EcoDXX1* systems were homologous, the restriction activity lacking in pES58 would be complemented by the *hsdR* gene products of the *EcoK* or *EcoB* system, resulting in restric-

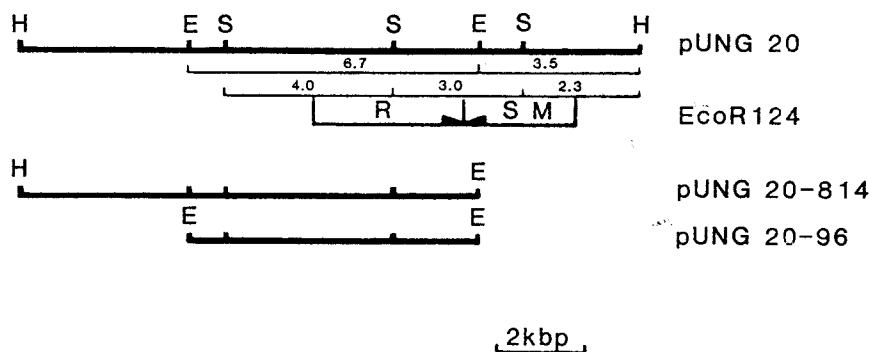


FIG. 4. Subclones derived from pUNG20 (Firman *et al.*, 1985) carrying *EcoR124* *hsd* genes (plasmids pUNG20-814 and pUNG20-96). The DNA fragments are drawn to scale and were derived by cloning an appropriate DNA fragment of pUNG20 into plasmid pUC8. Only the DNA fragments originated from pUNG20 are shown. The extent and order of the *hsdR*, *hsdS*, *hsdM* genes (Price *et al.* 1987) are indicated below the lines, showing the sizes of the pUNG20 fragments obtained after digestion with an appropriate restriction enzyme. The restriction sites are S, *Sal*I; H, *Hind*III; and E, *Eco*R1.

TABLE 3  
COMPLEMENTATION BETWEEN *EcoDXX1* AND *EcoR124* AND *EcoR124/3* *hsd* SYSTEMS

Strain	Genotype	Efficiency of plating of $\lambda$ phage						Phenotype
		$\lambda$ .K	$\lambda$ .R124	$\lambda$ .R124/3	$\lambda$ .R124.DXX1	$\lambda$ .R124/3.DXX1		
DH1	<i>hsdR</i> <sup>-</sup> <i>M</i> <sup>+</sup> <i>S</i> <sup>+</sup> (K)	1	1	1	1	1	Res <sup>-</sup> Mod <sup>+</sup> (K)	
DH1(R124, pES58)	<i>hsdR</i> <sup>+</sup> <i>M</i> <sup>+</sup> <i>S</i> <sup>+</sup> (R124)	10 <sup>-4</sup>	10 <sup>-2</sup>	10 <sup>-4</sup>	1	10 <sup>-4</sup>	Res <sup>+</sup> Mod <sup>+</sup> (R124)	
DH1(pUNG20, pES58)	<i>hsdR</i> <sup>+</sup> <i>M</i> <sup>+</sup> <i>S</i> <sup>+</sup> (DXX1)	10 <sup>-6</sup>	10 <sup>-3</sup>	10 <sup>-5</sup>	1	10 <sup>-5</sup>	Res <sup>+</sup> Mod <sup>+</sup> (DXX1)	
	<i>hsdR</i> <sup>+</sup> <i>M</i> <sup>+</sup> <i>S</i> <sup>+</sup> (R124)	10 <sup>-7</sup>	10 <sup>-7</sup>	10 <sup>-3</sup>	10 <sup>-7</sup>	1	Res <sup>+</sup> Mod <sup>+</sup> (R124/3)	
DH1(pUNG31, pES58)	<i>hsdR</i> <sup>+</sup> <i>M</i> <sup>+</sup> <i>S</i> <sup>+</sup> (DXX1)	10 <sup>-3</sup>	10 <sup>-3</sup>	10 <sup>-3</sup>	1	1	Res <sup>+</sup> Mod <sup>+</sup> (DXX1)	
DH1(pUNG20-814, pES58)	<i>hsdR</i> <sup>+</sup> <i>M</i> <sup>+</sup> <i>S</i> <sup>+</sup> (R124)	1	1	1	1	1	Res <sup>+</sup> Mod <sup>+</sup> (R124)	
DH1(pUNG20-96)	<i>hsdR</i> <sup>+</sup> <i>M</i> <sup>+</sup> <i>S</i> <sup>-</sup> (R124)	10 <sup>-3</sup>	10 <sup>-3</sup>	10 <sup>-3</sup>	1	1	Res <sup>+</sup> Mod <sup>-</sup> (DXX1)	
DH1(pUNG20-96, pES58)	<i>hsdR</i> <sup>+</sup> <i>M</i> <sup>+</sup> <i>S</i> <sup>-</sup> (R124)	10 <sup>-3</sup>	10 <sup>-3</sup>	10 <sup>-3</sup>	1	1	Res <sup>+</sup> Mod <sup>-</sup> (R124)	
	<i>hsdR</i> <sup>+</sup> <i>M</i> <sup>+</sup> <i>S</i> <sup>+</sup> (DXX1)	10 <sup>-3</sup>	10 <sup>-3</sup>	10 <sup>-3</sup>	1	1	Res <sup>+</sup> Mod <sup>+</sup> (DXX1)	

tion of both  $\lambda$ .K and  $\lambda$ .DXX1 phages. We also tested whether the DNA sequences coding for modification in the *EcoDXX1* system can complement this function in the *EcoK* and *EcoB* systems. In any case, we were not able to show complementation between these systems (data not presented).

In another set of experiments, complementation between *EcoDXX1* and plasmid-coded type I R-M systems was tested. Plasmid pES58 was introduced into strains carrying either the original plasmids R124, R124/3, and pYA15 or recombinant plasmids with the cloned *hsd* genes of the *EcoR124* or *EcoR124/3* system. In addition, we cloned into the pUC8 vector the *EcoRI* fragment of R124 DNA carrying only a *hsdR* gene of the *EcoR124* R-M system (plasmids pUNG20-96 and pUNG20-814) (Fig. 4). The results presented in Table 3 show that complementation did occur between *EcoDXX1* and *EcoR124* or *EcoR124/3* systems. To confirm the complementation between the gene products of these two systems, we introduced plasmids with the cloned *EcoRI* fragment of R124 DNA carrying only a functional *hsdR* gene into strain DH1 (pES58). As can be seen from the results in Table 3, the constructed strain DH1 (pES58, pUNG20-96) showed the ability to restrict phage  $\lambda$ . In the control experiments, complementation did not occur when we used the plasmid pCP1005::Tn1000 in which the *hsdR* gene of the *EcoR124* system was inactivated by the insertion of a transposon (data not shown). Complementation was also not observed between the *EcoDXX1* and the *EcoYA15* systems (data not presented).

#### Hybridization between Plasmid DNAs Carrying *EcoDXX1* and *EcoR124* *hsd* Genes

Because the *EcoDXX1* and *EcoR124* *hsd* genes showed complementation, Southern blotting experiments were performed to test for hybridization between the DNA molecules carrying cloned *hsd* genes. Restriction fragments of plasmid pUNG20 and pUNG20-96 DNAs were separated on an agarose gel, transferred to nitrocellulose, and probed with

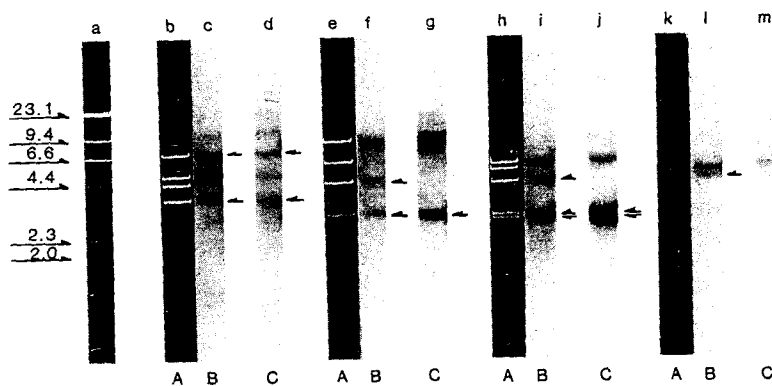


FIG. 5. Southern blotting of pUNG20 and pUNG20-96 DNAs probed with pES58 and pES7. The digested DNA was transferred from 1% agarose gel (A) to nitrocellulose filters and probed with  $^{32}$ P-labeled pES7 (B) and pES58 (C). Lanes b, e, and h show restriction fragments and lanes c, d, f, g, i, and j hybridization signals from pUNG20 DNA. Lane k shows restriction fragments and lanes l and m hybridization signals from pUNG20-96 DNA. Restriction enzymes used were *HindIII-EcoRI* (lanes b, c, d), *SalI* (lanes e, f, g, k, l, m), and *HindIII-SalI* (lanes h, i, j). Lane a shows  $\lambda$ CDNA digested with *HindIII* used as a molecular weight marker (kb). Arrows indicate fragments that are discussed in the text.

nick-translated pES58 or pES7 DNA. As shown in the previous sections, clone pES7 carries all three *hsd EcoDXX1* genes, while pES58 carries the *hsdM* and *S* genes and only a small part of the *hsdR* gene. Both the pES58 and pES7 probes hybridized to the 3.0-kb *SalI* fragment of pUNG20 (Fig. 5, lanes f, g, i, j) that is clearly within the *hsd* genes of the *EcoR124* R-M system (Fig. 4). Because this fragment of pUNG20 covers the *hsdR* and *hsdMS* genes, it is not possible to distinguish whether *hsdDXX1* shares a homology with all three *hsd* genes or only with a particular gene of this system. Probes pES58 and pES7 also hybridize to the 2.8-kb *HindIII-SalI* and 3.5-kb *HindIII-EcoRI* fragments of pUNG20 (Fig. 5, lanes c, d, i, j). These two fragments carry *hsdMS* genes of the *EcoR124* R-M system; however, it cannot be excluded that hybridization to these fragments reflects homology between the *EcoR124* sequences flanking the *hsd EcoR124* genes present in pUNG20 DNA (Fig. 4) and the plasmid sequences present in the probes. Similarly, the hybridization of probe pES7 to the 4.0-kb *SalI* fragments of pUNG20 and pUNG20-96 (Fig. 5, lanes f, i, l) could suggest homology between the *hsdR* genes of the *hsd EcoDXX1* and *EcoR124* systems, but again we cannot exclude that this

hybridization is between the plasmid sequences present in the *SalI* fragments of pUNG20 or pUNG20-96 (Fig. 4) and the plasmid sequences of the probe. Hybridization of probes pES58 and pES7 to the 6.7-kb *EcoRI* fragment of pUNG20 could suggest homology between *hsdR* and the *hsdS* genes of both *hsd* systems, but some problems with the presence of plasmid sequences preclude the drawing of final conclusions.

#### DISCUSSION

Biochemical characterization of the *EcoDXX1* restriction endonuclease predicted the existence of three genes, *hsdR*, *hsdM*, and *hsdS*, coding for the three protein subunits present in the native enzyme (Piekarowicz *et al.*, 1985). Cloning and subsequent subcloning of the pDXX1 DNA sequences coding for R-M properties of the *EcoDXX1* system showed that part of the 7.0-kb *BamHI-PstI* fragment located to the left end of the 15.5-kb *BamHI* fragment is necessary for a functional *hsdR* gene, while the modification and specificity genes must be coded for by the DNA sequences within a 7.0-kb *PstI-EcoRI* fragment located at the right of this fragment (Fig. 2). Using  $\lambda::Tn5$  transposon, we were

able to locate the *EcoDXX1* genes more precisely within the cloned pDXX1 fragment. We obtained a  $R^+M^+$ ,  $R^-M^+$ ,  $R^-M^-$  derivative of pES14 clones with Tn5 insertions in different places of the cloned R-M genes. Data from the transposon mutagenesis experiments showed that the restriction gene takes 3 kb, whereas the modification and specificity genes take 3–4 kb (Fig. 3). The relative molecular masses of the *EcoDXX1* proteins were estimated to be 98, 65, and 48 kDa (Piekawicz *et al.*, 1985), which corroborate with the sizes of the regions identified by Tn5 mutagenesis. Data obtained from the transposon mutagenesis of the cloned pES14 plasmid and those from the transposon mutagenesis of the *EcoR124* R-M system (Firman *et al.*, 1985; Price *et al.*, 1987) suggest that the sizes of the coding regions are similar in both cases (Figs. 3 and 4). Our data did not provide direct evidence on the order of the *hsdM* and *hsdS* genes characteristic for type I R-M systems, and further studies are necessary to determine this order. The preliminary observations of the unstable expression of the *hsdR* gene suggested that, similar to other type R-M systems, the *hsdR* gene is transcribed from an independent promoter other than that for the *hsdM* and *hsdS* genes; however, this unstable expression of the *hsdR* gene requires further studies.

The fact that the inability to transfer plasmids carrying the originally cloned *Bam*HI pDXX1 fragment to many *E. coli* strains can be abolished by a small deletion that has no effect on the expression of the restriction and modification properties suggests the existence of an additional gene(s) responsible for this effect. This gene could be, for example, analogous to the *kil* genes detected in several *E. coli* plasmids (Sabik *et al.*, 1983).

Two lines of evidence showed that the *hsd EcoDXX1* system shares great homology to two other type I R-M systems coded by the plasmid genes *EcoR124* and *EcoR124/3*. First, these three *hsd* systems can complement the missing functions of each other. On the other hand, we were unable to show complementation between *EcoDXX1* and chromosomally located R-M systems of *E. coli* which belong

to the type IA group represented in our studies by *EcoK* and *EcoB*. Because the *EcoR124* system is not related to the *EcoA* R-M system representing the type IB group, and *EcoDXX1* shows homology to the *EcoR124* R-M system, we can assume that the *EcoDXX1* R-M system is probably also not related to this group of type I R-M systems. We were also unable to detect complementation between *EcoDXX1* and *EcoYA15*. *EcoYA15* is probably a type I R-M system which is coded by plasmid genes (Piekawicz *et al.*, 1985; Piekawicz, unpublished work). Second, hybridization data showed great homology between all three genes of the *EcoDXX1* and *EcoR124* systems, which together with the transposon mutagenesis data of both *hsd* regions clearly show the similarity in the genetic organization of both R-M systems. This is also strengthened by the fact that in these two systems (as well as in *EcoR124/3*) the presence of additional regions located outside the *hsd* genes plays a role in the control of R-M genes (Firman *et al.*, 1983, 1985; E. Skrzypek, unpublished results).

The biochemical properties of the *EcoDXX1* restriction enzyme (Piekawicz *et al.*, 1985; Piekawicz and Goguen, 1986) and the results presented in this paper clearly show that the *EcoDXX1* R-M system belongs to the IC group of type I R-M systems of *E. coli* and *Salmonella* represented so far by two plasmid-borne systems, *EcoR124* and *EcoR124/3*.

#### ACKNOWLEDGMENTS

We thank S. W. Glover and C. Price for pUNG20, pUNG31, and pCP1005::Tn1000 plasmids and S. Iida for  $\lambda$ ::Tn5 transposon. We thank R. Yuan and D. Stein for critically reviewing this manuscript. This work was supported by Polish Academy of Sciences Grant 3.13.

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Communicated by Richard D. Kolodner