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Occurrence of Small Hsd Plasmids in *Salmonella typhi*, *Shigella boydii*, and *Escherichia coli*

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The natural occurrence of small Hsd (host specificity for DNA) plasmids was demonstrated in restriction endonuclease-producing strains of *Salmonella typhi*, *Shigella boydii*, and *Escherichia coli*. The five Hsd plasmids isolated were between 5.0 and 12.2 kilobases long. The copy number of all the Hsd plasmids was high (more than 10 copies per cell). Introduction of these small plasmids into *E. coli* strain 0 drastically lowered the efficiency of plating of the λ.0 phages (the efficiency of plating was less than 5×10^{-5} PFU^{-1}). High restriction endonuclease activities were detected in the Hsd plasmid-positive strains because of the elevated copy numbers of the *hsdR*$^+$ gene. The advantages of using *E. coli* strains containing the small Hsd plasmids for purification of type II restriction endonucleases are discussed.

It has been widely accepted that much of recent biological technology (e.g., structural analysis and cloning of DNA) is highly dependent on the action of type II restriction endonucleases. To date, approximately 100 different restriction endonucleases have been purified from various bacterial strains (28). Among these enzymes, the stable *Escherichia coli* restriction endonucleases, such as *Eco*RI, are the most useful because they are easy to purify and therefore are less expensive. In *E. coli*, some of the structural genes of restriction endonucleases are known to be located on a plasmid, while the others are on chromosomal DNA (3). The Hsd (host specificity for DNA) plasmids are a group of plasmids which carry genes for restriction endonucleases and modification methylase (3). The Hsd plasmid which produces endonucleases *Eco*RI, *Eco*RII, *Eco*RV, and *Eco*VIII in *E. coli* has been investigated in detail by workers in various laboratories. The *Eco*RI and *Eco*RII genes are carried on a large R plasmid with conjugal transfer ability (29, 33), while the *Eco*VIII gene is on a small 4.4-kilobase (kb) plasmid without drug resistance genes and conjugal transfer ability (23). The *Eco*RV gene was first believed to be carried on a kanamycin-resistant plasmid with conjugal transfer ability (14), but later was shown to be located on a small 6.2-kb plasmid without kanamycin resistance and conjugal transfer ability (8).

The occurrence of two small Hsd plasmids in *E. coli* led us to investigate the possibility that the small Hsd plasmids might be widespread in the *Salmonella-Shigella-E. coli* group. Using eight type II restriction endonuclease-producing strains from our laboratory, we investigated the widespread occurrence of the small Hsd plasmids in the *Salmonella-Shigella-E. coli* group, as well as the usefulness of the small Hsd plasmids for production of restriction endonucleases.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. The bacterial strains which we used are listed in Table 1. Bacteriophages P1Cm1, ViII, and P22Cm1 have been described else where (12, 21, 22); λ and P1 were obtained from W. Arber. The plasmids used were pBR322 (Ap^r Tc^r Hsd$^-$) (7), S-a (Cm^r Sm^r Su^r Km^r Hsd$^-$) (31), pSC101 (Tc^r Hsd$^-$) (10), RY13 (Cm^r Sm^r Su^r Ap^r Hsd$^+$) (33), N3 (Sm^r Su^r Tc^r Hsd$^+$) (31), and pJA4620 (Tc^r Hsd$^+$) (2).

Media and chemicals. The media which we used were Penassay broth (Difco Laboratories, Detroit, Mich.) and Penassay agar for growth and titration of bacteria and L broth (19) and L agar for phages. Polypeptone (Daigo Eiyo Chemical Co., Tokyo, Japan) was used instead of tryptone (Difco) in L broth. The following antibiotics were used: ampicillin, kanamycin, and streptomycin from Meiji-Seika Co., Tokyo, Japan; chloramphenicol from Sankyo Co., Tokyo, Japan; and tetracycline from Lederle Laboratories, Pearl River, N.Y.

Growth and titration of phage. The methods of Mise and Arber (22) were used for phage growth and titration.

Isolation of plasmid DNAs and measurement of plasmid size and copy number. Plasmids carried in *Salmonella*, *Shigella*, and *E. coli* were isolated by alkaline lysis (18, 20). Agarose gel electrophoresis of plasmid DNA was performed as described by Tanaka et al. (30). The following phage and plasmid DNAs were used as size markers: ϕX174 replicative-form DNA and phage λ DNA from New England BioLabs, Inc., Beverly, Mass.; simian virus 40 DNA from Bethesda Research Laboratories, Inc., Gaithersburg, Md.; and pBR322 DNA, which was a gift from K. Nakajima, Tokyo University, Tokyo, Japan. The copy number of each Hsd plasmid was determined by using the ethidium bromide staining method described by Broach (9); the intensity of the ethidium bromide staining of an Hsd plasmid was compared with the intensity of staining of a coexisting plasmid whose molecular weight and copy number were known.

Method used for isolation of Hsd$^-$ mutants from Hsd$^+$ strains. Hsd$^-$ mutants were easily obtained from the original Hsd$^+$ strain by phage P1Cm or P22Cm transduction, followed by curing of the phage genome as described previously (23, 32). This experiment was carried out with the expectation that phage P1Cm or P22Cm could be transduced to Hsd$^-$ mutants at a much higher frequency than the original Hsd$^+$ strain; thus, the Hsd$^-$ mutants could be easily detected among Cam^r transductants.

Method used for transformation of Hsd plasmids. Hsd

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TABLE 1. Bacterial strains used

Strain	Properties[a]	Source or reference(s)
Restriction endonuclease-producing strains		
Salmonella typhi 27	*Sty*I$^+$, ViII phage type 27, Cam^s Str^r Tet^s Kan^s Amp^s	1
Shigella boydii 13	*Sbo*13$^+$, Cam^s Str^s Tet^s Kan^s Amp^s	NCTC 9361
E. coli H304	*Eco*O34$^+$, O34:K(−):H10, Cam^s Str^s Tet^s Kan^s Amp^s	26
E. coli K11a	*Eco*O65$^+$, O65:K(−):H(−), Cam^s Str^s Tet^s Kan^s Amp^s	26
E. coli H709c	*Eco*O109$^+$, O109:K(−):H19, Cam^s Str^s Tet^s Kan^s Amp^s	26
E. coli TB14	*Eco*T14$^+$, Cam^s Str^r Tet^r Kan^r Amp^r	Supplied by N. Terakado
E. coli TB22	*Eco*T22$^+$, Cam^s Str^s Tet^r Kan^s Amp^s	Supplied by N. Terakado
E. coli TH38	*Eco*T38$^+$, Cam^s Str^r Tet^s Kan^r Amp^s	Supplied by N. Terakado
E. coli E1585-68	*Eco*VIII$^+$, O156:K(−):H47, Cam^s Str^s Tet^s Kan^s Amp^s	26, 27
Restriction endonuclease-negative strains		
Salmonella typhi A	0 strain of *Salmonella typhi*	1
Salmonella typhi 27Hsd$^-$	Hsd$^-$ mutant of *Salmonella typhi* 27	This study
E. coli K-12 WA921	*hsdR*$^-$, *E. coli* K-12 strain 0, Cam^s Str^s Tet^s Kan^s Amp^s	33
E. coli B YY201	*hsdB*$^-$ *hsdRI*$^-$ mutant of RY13 (31), Cam^r Str^r Tet^s Kan^s Amp^r	Isolated in our laboratory
E. coli B HB101	*hsdB*$^-$ *recA13*, strain 0, Cam^s Str^s Tet^s Kan^s Amp^s	16
E. coli C	Wild type, strain 0, Cam^s Str^s Tet^s Kan^s Amp^s	Supplied by H. Ikeda

[a] For strain 0, see reference 4.

plasmids were transformed as described by Mise and Nakajima (23). Briefly, the Hsd plasmids were transformed into Ca^{2+}-treated cells along with the R plasmid, and the resulting antibiotic-resistant transformants were purified on antibiotic-containing agar. In each experiment, R-plasmid DNA (0.2 to 0.3 μg) and Hsd plasmid DNA (0.2 to 1.0 μg) were mixed with 2×10^7 $CaCl_2$-treated *E. coli* cells in the standard transformation buffer (13). Under these conditions, the ratio of the number of the plasmid molecules to the number of *E. coli* cells in the buffer was kept at more than 20. Therefore, both plasmid DNAs were expected to be incorporated into competent cells. The occurrence of Hsd plasmids in the R$^+$ transformants was tested by the cross-streaking method for identification of Hsd$^+$ strains described by Wood (32).

Restriction analysis. Restriction analysis was performed as described by Mise and Nakajima (23). TA buffer (25) was used for the restriction reactions.

RESULTS

Initial characterization of Hsd$^+$ strains and their restriction endonucleases. The restriction endonuclease-producing strains which we investigated are listed in Table 1. These strains were selected from about 800 strains of *Salmonella*, *Shigella*, and *E. coli* (Mise, unpublished data). *Salmonella typhi* 27 and *Shigella boydii* 13 are standard type strains that are widely used by bacteriologists. *E. coli* H304, K11a, H709c, and E1585-68 were antigenic tester strains of the *E. coli* O group. *E. coli* TB14, TB22, and TH38 were isolated from swine by N. Terakado (The National Institute of Animal Health of Japan). All of the strains grew well in enriched media such as Penassay broth and L broth and were resistant to phages P1 and λ. Five of the nine strains (*Shigella boydii* 13 and *E. coli* H304, K11a, H709c, and E1585-68) were sensitive to ampicillin, chloramphenicol, kanamycin, streptomycin, and tetracycline at concentrations of 25 μg/ml, while the remaining four strains (*Salmonella typhi* 27 and *E. coli* TB14, TB22, and TH38) were resistant to at least one of the five antibiotics (Table 1). Each strain exhibited a high restriction endonuclease activity. The restriction endonuclease produced by these strains are shown in Table 1. Five of the endonucleases (*Sty*I, *Sbo*13, *Eco*T22, *Eco*O109, and *Eco*VIII) were purified without contaminating nuclease activity. The remaining four endonucleases (*Eco*T14, *Eco*T38, *Eco*O34, and *Eco*O65) were not completely purified and contained small amounts of contaminating nucleases. The cleavage patterns of λ DNA obtained with the purified endonucleases are shown in Fig. 1. These patterns were clearly different from each other and from those obtained with endonucleases *Eco*RI, *Eco*RII, and *Eco*RV (for the cleavage patterns of λ DNA with *Eco*RI, *Eco*RII, and *Eco*RV, see the 1985–86 catalog of New England BioLabs, Inc., and reference 28). Preliminary experiments with partially purified endonucleases indicated that *Eco*T14 was an isoschizomer of *Sty*I and that the recognition sequences of *Eco*O34, *Eco*T38, and *Eco*O65 seemed to be different from each other and from those of five purified endonucleases (data not shown). Therefore, the nine endonucleases fell into eight different specificity groups.

Small Hsd plasmid in *Salmonella typhi* 27. *Salmonella typhi* can be divided into about 100 phage types on the basis of host range or host-controlled variation of phage ViII or both (1, 12, 15). While screening restriction endonuclease-producing strains in the standard phage type strains of *Salmonella typhi*, we found that *Salmonella typhi* 27 is a good producer of a restriction endonuclease; more than 10^4 U of restriction endonuclease *Sty*I was released from 1 g of *Salmonella typhi* 27 cells by EDTA-lysozyme treatment of rapidly growing cells (11). It has been reported previously by two groups of workers that *Salmonella typhi* 27 exhibits host-controlled restriction against phage ViII.0 (1, 6). Mutants of *Salmonella typhi* 27 which did not exhibit host-controlled restriction and modification (*Salmonella typhi* 27Hsd$^-$) were obtained from the original *Salmonella typhi* 27 culture by P22 phage transduction, followed by curing of the phage genome as described in Materials and Methods. By using the alkaline lysis method (20), we showed that the original *Salmonella typhi* 27 culture contained a 5.0-kb plasmid designated pST27, while Hsd$^-$ mutants lacked this small plasmid (Fig. 2, lanes 1 and 2). This finding strongly suggested that plasmid pST27 DNA is the Hsd plasmid. To confirm this, plasmid pST27 DNA was purified from *Salmonella typhi* 27 and transformed into *E. coli* WA921 along with plasmid S-a (Table 2, experiment 1). The resulting Hsd$^+$ transformants simultaneously received plasmid pST27 and the ability to produce endonuclease *Sty*I (Fig. 2, lane 4). Plasmid pST27 was again transformed from strain WA921

(pST27) into strain YY201 and finally from strain YY201(pST27) into *Salmonella typhi* A (Table 2, experiments 2 and 3). All of the strains carrying plasmid pST27 not only exhibited host-controlled restriction against phage λ or ViII, but also produced endonuclease *Sty*I at high levels (Fig. 2) (Mise, unpublished data). Although the frequency of transformation of the plasmid from *Salmonella typhi* to *E. coli* or from *E. coli* strain to *E. coli* strain was high, the frequency of transformation from *E. coli* to *Salmonella typhi* was extremely low (Table 2). The reason for this is not known.

Salmonella typhi A which received plasmid pST27 exhibited essentially the same patterns of host-controlled variation as *Salmonella typhi* 27 (Table 3). The phage Vi typing method developed by Anderson and Fraser (1) indicated that *Salmonella typhi* A(pST27) and *Salmonella typhi* 27Hsd$^-$ were identical to *Salmonella typhi* 27 and *Salmonella typhi* A, respectively. These results clearly indicated that plasmid pST27 is a type-determining plasmid in *Salmonella typhi*. It is of some interest that plasmid pST27 reduced the plaque-forming ability of phage ViII.0 to 9×10^{-9} PFU^{-1}.

Small Hsd plasmid in *Shigella boydii*. *Shigella boydii* 13 exhibited high activity of a restriction endonuclease designated *Sbo*13 (more than 2×10^3 U/g of wet cells) with restriction fragments of λ DNA identical to those produced by endonuclease *Nru*I (Fig. 1, lane 2). Agarose gel electrophoresis of plasmid DNA isolated from *Shigella boydii* 13 revealed the presence of several different plasmid DNA species (14.0, 10.6, 7.1, 5.6, 4.8, and 1.7 kb) (Fig. 3, lane 1). This plasmid mixture was transformed into strain YY201

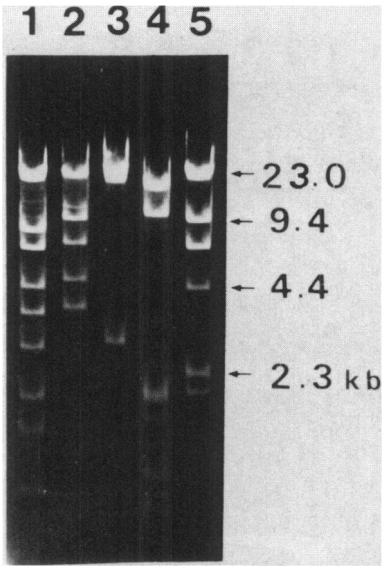

FIG. 1. Cleavage patterns of phage λ DNA after digestion with various restriction endonucleases from *Salmonella typhi*, *Shigella boydii*, and *E. coli*. The digests were analyzed on 1% agarose gels at 80 V. Lane 1, *Sty*I (from *Salmonella typhi* 27); lane 2, *Sbo*13 (*Shigella boydii* 13); lane 3, *Eco*O109 (*E. coli* H709c); lane 4, *Eco*T22 (*E. coli* TB22); lane 5, *Eco*VIII (*E. coli* E1585-68). The method used to purify all of these restriction endonucleases except *Eco*VIII and *Sty*I (23, 24) will be described elsewhere (Mise and Nakajima, submitted). Restriction endonucleases *Sbo*13, *Eco*T22, and *Eco*VIII are isoschizomers of *Nru*I, *Ava*III, and *Hin*dIII, respectively, while *Eco*O109 and *Sty*I recognize and cleave new sequences that have not been described previously (23, 24; Mise and Nakajima, submitted). The values indicate the sizes of fragments.

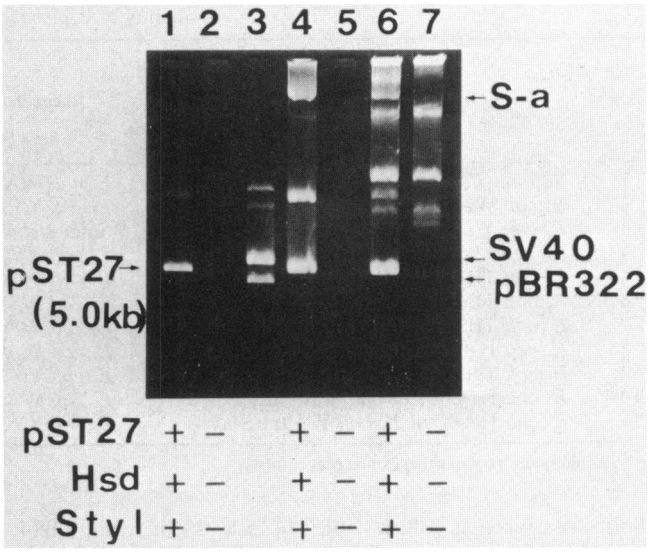

FIG. 2. Occurrence of the 5.0-kb Hsd plasmid (pST27) in *Sty*I endonuclease-producing cells. Plasmid DNA was isolated from the strains by the alkaline lysis method of Maniatis et al. (20). Agarose (1%) gel electrophoresis of the DNAs was carried out at 80 V for 150 min. Lane 1, *Salmonella typhi* 27; lane 2, *Salmonella typhi* 27Hsd$^-$; lane 3, simian virus 40 (SV40) 5.2-kb and pBR322 4.4-kb size standards; lane 4, *E. coli* WA921 (pST27, S-a); lane 5, *E. coli* WA921; lane 6, *E. coli* YY201(pST27, S-a); lane 7, *E. coli* YY201. The occurrence of host specificity for DNA (Hsd) in these strains was tested by the method of Wood (32), using phage λ.0 for *E. coli* strains and phage ViII.0 for *Salmonella typhi* strains. We noted that all of the strains carrying the 5.0-kb plasmid had Hsd$^+$ characteristics and produced endonuclease *Sty*I (StyI$^+$). Although no clear bands were observed in lanes 2 and 5, these cells certainly lysed, since a faint band of chromosomal DNA migrating slightly faster than the plasmid S-a band was observed.

along with plasmid S-a, and R$^+$ transformants were selected. As shown in Table 2 (experiment 4), Hsd$^+$ transformants were detected at a high frequency (0.39/Kan^r transformant). The 5.6-kb plasmid of *Shigella boydii* 13 was found in all 11 Hsd$^+$ transformants, while the other plasmids of *Shigella boydii* were absent in some of the transformants (Fig. 3, lanes 2, 5, and 6). Our results clearly indicated that the small 5.6-kb plasmid designated pSB13 is the Hsd plasmid of *Shigella boydii* (13). The copy number of plasmid pSB13 was high (more than 20 copies per cell).

Small Hsd plasmids in *E. coli*. We have shown previously that *E. coli* E1585-68 carries a multicopy 4.4-kb Hsd plasmid (23). In order to confirm the widespread occurrence of small Hsd plasmids in *E. coli* insertion plasmid DNAs were isolated from six restriction endonuclease-producing strains of *E. coli* (Table 1). All of the strains carried at least one species of plasmid DNA. The plasmid mixture was transformed into *E. coli* strain 0 along with the R plasmid, and we searched for Hsd$^+$ transformants among the R$^+$ transformants. As shown in Table 2 (experiments 5 through 11), Hsd$^+$ transformants were detected among the R$^+$ transformants in *E. coli* TB14, TB22, and H304, while Hsd$^+$ transformants were not detected in *E. coli* K11a, H709c, or TH38 in spite of several trials. Agarose gel electrophoresis of plasmid DNAs isolated from the Hsd$^+$ transformants (Fig. 4) indicated that the *hsd*$^+$ gene was carried on small plasmids in all of the restriction endonuclease-producing cells. The copy number of these Hsd plasmids was always more than 10 copies per cell. The designations of these Hsd plasmids and

TABLE 2. Cotransformation of R plasmids and Hsd plasmids to $CaCl_2$-treated 0 strains

Expt	Origin of donor plasmid DNA	Recipient strain	Selective marker for transformation of R plasmid	Frequency of transformation (no. of transformants per μg of R-plasmid DNA)	No. of Hsd^+ transformants/no. of R^+ transformants tested
1	*Salmonella typhi* 27 + *E. coli* WA921(S-a)	*E. coli* WA921	Cm^r	3×10^3	2/100 (2)[a]
2	*E. coli* WA921(pST27, S-a)	*E. coli* YY201	Km^r	2×10^3	12/20 (60)
3	*E. coli* YY201 (pST27, S-a)	*Salmonella typhi* A	Cm^r	3	1/3 (33)
4	*Shigella boydii* 13 + *E. coli* WA921(S-a)	*E. coli* YY201	Km^r	1×10^3	28/72 (39)
5	*E. coli* TB14 + *E. coli* WA921(S-a)	*E. coli* WA921	Cm^r	<5	
6	*E. coli* TB14 + pBR322 DNA	*E. coli* WA921	Tc^r	1×10^3	5/160 (3)
7	*E. coli* TB22 + *E. coli* WA921(S-a)	*E. coli* WA921	Cm^r	8×10^3	14/132 (11)
8	*E. coli* H304 + *E. coli* WA921(S-a)	*E. coli* WA921	Cm^r	8×10^2	7/96 (7)
9	*E. coli* TH38 + *E. coli* WA921(S-a)	*E. coli* YY201	Km^r	8×10^2	0/318 (<0.3)
10	*E. coli* K11a + *E. coli* WA921(S-a)	*E. coli* WA921	Cm^r	2×10^2	0/177 (<0.6)
11	*E. coli* H709c + *E. coli* WA921(S-a)	*E. coli* YY201	Km^r	2×10^3	0/354 (<0.3)

[a] The numbers in parentheses are percentages.

their sizes were as follows: pEC14 (5.8 kb) in strain TB14; pEC22 (12.2 kb) in strain TB22; and pEC34 (5.3 or 7.3 kb or both) in strain H304 (Fig. 4). Two species of plasmid DNA were detected in all seven strain H304 Hsd^+ transformants (Table 2, experiment 8); we assumed that at least one of these species was the Hsd plasmid.

Host specificity for DNA produced in *E. coli* strain 0 carrying small Hsd plasmids. Table 4 shows the results of efficiency-of-plating (EOP) experiments for λ variant phages. Our data demonstrated that all of the host specificities except those for pST27 and pEC14 were mutually exclusive to each other and to RI, RII, and pJA4620 host specificities. It is no surprise that the pST27 and pEC14 host specificities were identical, since these plasmids produced isoschizomers (see above).

The levels of restriction exerted by the small Hsd plasmids were generally very high; the EOP of unmodified λ · 0 was less than 5×10^{-5} PFU^{-1} on *E. coli* strain 0 carrying the Hsd plasmid. The exception of this was the level of restriction by pEC156; the EOP of λ · 0 was only 10^{-2} PFU^{-1} on *E. coli* (pEC156), as described previously (23). All of the *E. coli* strains with the small plasmid exhibited high restriction endonuclease activities (data not shown).

DISCUSSION

Table 5 summarizes the results of the experiments on Hsd plasmids described in this paper. The natural occurrence of the small Hsd plasmids in the *Salmonella-Shigella-E. coli* group is clearly shown. Until recently, the role of most small plasmids in members of the *Enterobacteriaceae* has been obscure; exceptions were the ColI type plasmids and the sulfonamide-resistant plasmids (5, 17). Our results indicate that some cryptic plasmids carry an *hsd*$^+$ gene, the products of which (restriction endonucleases) protect against infection by bacteriophages.

Although no Hsd plasmid was detected in *E. coli* H709c, K11a, or TH38 (Table 2, experiments 9 through 11), it is still possible that the *hsd*$^+$ gene of these three strains is carried on a plasmid since all of the strains carry at least one species of plasmid DNA. Moreover, it is possible that introduction of the Hsd plasmid into the host cells by transformation might be lethal to the host cell. It is possible that a restriction

TABLE 3. EOP of ViII variant phages on *Salmonella typhi* strains[a]

Variant phage	EOP (PFU^{-1}) on the following host strains:			
	Salmonella typhi A	*Salmonella typhi* A(pST27)[b]	*Salmonella typhi* 27	*Salmonella typhi* 27 Hsd^-
ViII·A or ViII·A·(pST27)·A	1.0	9×10^{-9}	3×10^{-9}	0.9
ViII·A(pST27)	1.0	0.9	0.8	0.9
ViII·27	1.0	1.0	0.7	0.8
ViII·27Hsd^-	1.0	8×10^{-9}	2×10^{-9}	0.7

[a] The values are means of two independent determinations.

[b] *Salmonella typhi* A(pST27) was constructed by transforming plasmid pST27 into $CaCl_2$-treated *Salmonella typhi* A (Table 2, experiment 3).

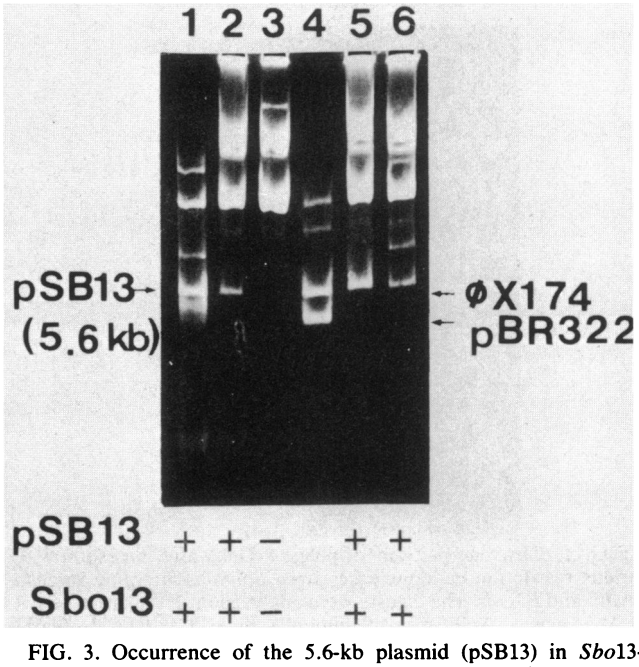

FIG. 3. Occurrence of the 5.6-kb plasmid (pSB13) in *Sbo*13-producing cells. The method used for isolation of plasmid DNA was the method described in the legend to Fig. 2. Agarose (1%) gel electrophoresis of the DNAs was carried out at 80 V for 150 min. Lane 1, *Shigella boydii* 13; lanes 2, 5, and 6, *E. coli* YY201(pSB13, S-a); lane 3, *E. coli* YY201; lane 4, ϕX174 5.4-kb and pBR322 4.4-kb DNA size standards. The three *E. coli* YY201(pSB13, S-a) strains in lanes 2, 5, and 6 represented the Hsd^+ transformants described in Table 2 (experiment 4).

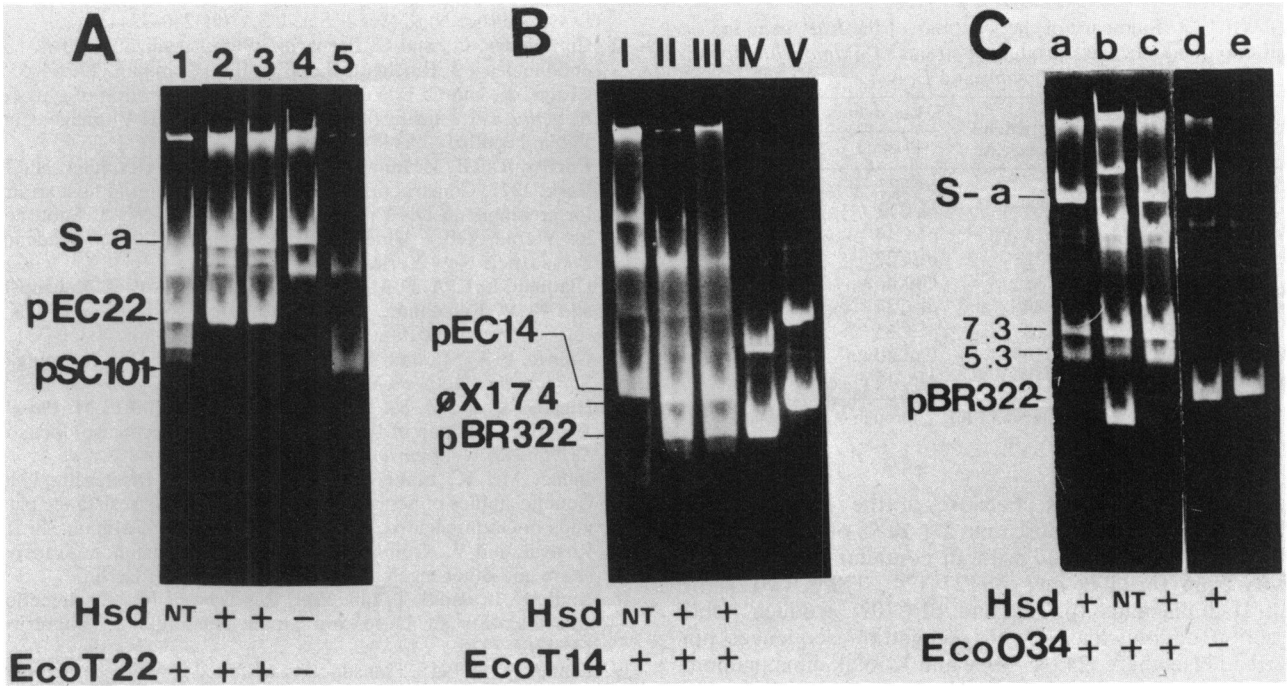

FIG. 4. Occurrence of the small Hsd plasmids in *E. coli*. The methods used to determine Hsd characteristics and to isolate plasmid DNA were the methods described in the legend to Fig. 2. Agarose (1%) gel electrophoresis of the DNAs was carried out at 80 V for 150 to 180 min. (A). Occurrence of the 12.2-kb plasmid (pEC22) in *Eco*T22 endonuclease-producing cells. Lane 1, *E. coli* TB22; lane 2, *E. coli* WA921(pEC22, S-a); lane 3, *E. coli* HB101(pEC22, S-a); lane 4, an Hsd$^-$ mutant derived from *E. coli* WA921(pEC22, S-a); lane 5, plasmid pSC101 8.5-kb size standard. (B) Occurrence of the 5.8-kb plasmid (pEC14) in *Eco*T14 endonuclease-producing cells. Lane I, *E. coli* TB14; lane II, *E. coli* WA921(pEC14, pBR322); lane III, *E. coli* HB101(pEC14, pBR322); lane IV, plasmid pBR322 4.4-kb DNA size marker; lane V, ϕX174 5.4-kb replicative-form DNA size marker. (C) Occurrence of 5.3- and 7.3-kb plasmids in *Eco*O34-producing cells. Lane a, *E. coli* WA921(pEC34, S-a); lane b, *E. coli* H304; lane c, *E. coli* HB101(pEC34, S-a); lane d, *E. coli* HB101(pEC156, S-a); lane e, plasmid pBR322 size marker. The values indicate size (in kilobases). NT, Not tested.

endonuclease produced from a newly introduced Hsd plasmid would cleave the host DNA, and very few transformants could then be detected. Strain H709c carries three species of small plasmid DNA (2.4, 3.0, and 7.2 kb), while strains K11a and TH38 carry one large plasmid. The *hsd*$^+$ gene of strain K11a might be carried on a large (36-kb) plasmid since all four Hsd$^-$ mutants of strain K11a lacked this plasmid (Yoshida, unpublished data). No data suggesting the location of the *hsd*$^+$ gene of strain TH38 have been obtained.

The occurrence of the small Hsd plasmids in the *Salmonella-Shigella-E. coli* group is useful for production of restriction endonucleases and for studies on the mechanisms of host-controlled variation for the following reasons. First, the small Hsd plasmids are easy to handle; many of them can be transformed into nonpathogenic *E. coli* K-12 or B along with an R plasmid as described above. Second, the small Hsd plasmids from *Salmonella typhi* and *Shigella* produce large amounts of restriction endonucleases in *E. coli*, as well

TABLE 4. EOP of λ variant phages on *E. coli* 0 strains carrying various Hsd plasmids[a]

Variant phage	EOP (PFU$^-$) on *E. coli* strain 0 carrying:[b]									
	No plasmid	pST27	pSB13	pEC14	pEC22	pEC34	pEC156	RY13	N3	pJA4620
λ·	1	5×10^{-6}	1×10^{-5}	4×10^{-5}	2×10^{-7}	3×10^{-7}	1×10^{-2}	2×10^{-4}	2×10^{-2}	2×10^{-4}
λ·pST27	1	1	3×10^{-5}	1	2×10^{-7}	4×10^{-7}	1×10^{-2}	2×10^{-4}	2×10^{-2}	3×10^{-4}
λ·pSB13	1	3×10^{-6}	1	2×10^{-5}	2×10^{-8}	1×10^{-7}	2×10^{-2}	2×10^{-4}	2×10^{-2}	2×10^{-4}
λ·pEC14	1	1	2×10^{-5}	1	2×10^{-7}	2×10^{-7}	1×10^{-2}	3×10^{-4}	3×10^{-2}	3×10^{-4}
λ·pEC22	1	3×10^{-5}	3×10^{-5}	5×10^{-5}	1	3×10^{-7}	2×10^{-2}	5×10^{-4}	3×10^{-2}	3×10^{-4}
λ·pEC34	1	1×10^{-5}	3×10^{-5}	4×10^{-5}	2×10^{-7}	1	1×10^{-2}	5×10^{-4}	3×10^{-2}	2×10^{-4}
λ·pEC156	1	1×10^{-5}	3×10^{-5}	2×10^{-5}	2×10^{-7}	3×10^{-7}	1	5×10^{-4}	2×10^{-2}	3×10^{-4}
λ·RY13	1	4×10^{-6}	1×10^{-5}	3×10^{-5}	2×10^{-7}	4×10^{-7}	1×10^{-2}	1	NT[c]	NT
λ·N3	1	4×10^{-6}	1×10^{-5}	4×10^{-5}	3×10^{-7}	3×10^{-7}	1×10^{-2}	NT	1	NT
λ·pJA4620	1	5×10^{-6}	1×10^{-5}	4×10^{-5}	6×10^{-7}	4×10^{-7}	1×10^{-2}	NT[c]	NT	1

[a] The values are means of two independent determinations. The host strains of the Hsd plasmids used were strain YY201 for plasmid pSB13 and strain WA921 for all of the other plasmids. Both strain YY201 and strain WA921 are 0 strains.

[b] Plasmids RY13 and N3 produce endonucleases *Eco*RI and *Eco*RII, respectively. Although pJA4620 confers host specificity for DNA on the host, no restriction endonuclease has been isolated from pJA4620$^+$ strains.

[c] NT, Not tested.

TABLE 5. Summary of the locations of the *hsd*$^+$ gene in restriction endonuclease-producing strains of *Salmonella typhi*, *Shigella boydii*, and *E. coli*

Strain	Restriction endonuclease	Location of the *hsd*$^+$ gene[a]	
		Plasmid	Size (kb)
Salmonella typhi 27	*Sty*I	pST27	5.0
Shigella boydii 13	*Sbo*13	pSB13	5.6
E. coli TB14	*Eco*T14	pEC14	5.8
E. coli TB22	*Eco*T22	pEC22	12.2
E. coli TH38	*Eco*T38	Unknown	
E. coli H304	*Eco*O34	pEC34	5.3 and/or 7.3
E. coli K11a	*Eco*O65	pEC65	36
E. coli H709c	*Eco*O109	Unknown	
E. coli E1585-68	*Eco*VIII	pEC156	4.4[b]

[a] Deduced from experiments shown in Fig. 2 through 4 and Table 2.
[b] Data from reference 23.

as in the original hosts, because of the elevated copy numbers. For example, more than 2×10^3 U of endonuclease *Sty*I activity were purified without contaminating nuclease activity from 1 g of *E. coli* (pST27) (24). Third, two of the small Hsd plasmids (pST27 and pEC109) produce noble restriction endonucleases with recognition sequences not described elsewhere (24; K. Mise and K. Nakajima, submitted for publication). Moreover, these two endonucleases, as well as *Eco*T22, *Sbo*13, and *Eco*VIII, were stable during purification procedures. Therefore, the occurrence of small Hsd plasmids in the *Salmonella-Shigella-E. coli* group should facilitate purification of various type II restriction endonucleases.

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