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A Basic Replicon of Virulence-Associated Plasmids of *Shigella* spp. and Enteroinvasive *Escherichia coli* Is Homologous with a Basic Replicon in Plasmids of IncF Groups

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Shigella **species and enteroinvasive *Escherichia coli* strains carry a large (120- to 140-megadalton) plasmid called pINV, which contains genes essential for the invasiveness of these pathogens. Hybridization with specific probes derived from the RepFIC and RepFIB replicons of the IncF1 Ent plasmid P307 showed that pINVs present in 35 clinical isolates are homologous with RepFIC but not RepFIB, regardless of the serogroup of the *Shigella* or *E. coli* strain. RepFIC of P307, in turn, is very similar to RepFIIA replicons of IncFII R plasmids. These and other related replicons constitute the RepFIIA family. With one pINV, pWR110, a plasmid of *Shigella flexneri* 5, we demonstrated the existence of a functional replicon, RepINV, with a restriction map similar to that of RepFIIA of plasmid R1. We isolated the putative *inc* RNA coding region of RepINV, which is a major determinant of incompatibility. The nucleotide sequence of the RepINV-*inc* RNA-coding region was determined and compared with the corresponding sequences of RepFIC and RepFIIA. The differences were small, but apparently were sufficient to affect the target specificity of the *inc* RNAs, thus rendering the replicons compatible with each other. We conclude that pINVs present in *Shigella* spp. and enteroinvasive *E. coli* constitute a homogeneous group, containing one basic replicon that belongs to the RepFIIA family of replicons.**

The ability of *Shigella* spp. and enteroinvasive *Escherichia coli* (EIEC) to enter epithelial cells and cause disease (5) depends on the presence of several chromosomal genes and a large plasmid (4, 18–20, 23, 24), which we shall refer to as pINV. In regard to their role in pathogenicity, pINVs of different *Shigella* and EIEC strains seem to be functionally equivalent. However, besides this common characteristic, no other features have been used to identify these plasmids as a group. Although Sansonetti et al. (19) assigned a pINV plasmid present in *Shigella sonnei* to incompatibility group IncFI on the basis of its incompatibility with IncFI plasmid R386, the incompatibility behavior of these plasmids is generally unknown.

Plasmids that produce F-type pili have been classified into six incompatibility groups, IncFI to IncFVI. Many of these plasmids contain more than one basic replicon, a DNA segment about 2 kilobase pairs (kbp) long with an origin of replication and associated control genes. Thus, IncFI plasmids may contain as many as three basic replicons in different regions of the plasmid, which have been denoted RepFIA, RepFIB, and RepFIC (2, 15). The above-mentioned plasmid R386 possesses functional RepFIB and RepFIC, but only part of RepFIA, which is associated with partitioning (13). Most plasmids belonging to other IncF groups have basic replicons that are homologous with RepFIC. An example is the RepFIIA basic replicon in IncFII plasmids, and since this was the first basic replicon of this type to be analyzed, the group of homologous replicons is referred to as the RepFIIA family (16). In contrast to plasmids belonging to other IncF groups, IncFII plasmids appear to contain only one basic replicon.

During characterization of pWR110, a pINV plasmid present in strain M90T of *S. flexneri* 5 (20), it was shown that this plasmid has a functional replicon homologous with RepFIC. It therefore became of interest to see whether the homology with RepFIC is a common feature of pINVs; it could then be used as a criterion for characterizing these plasmids.

The results reported here show that pINV plasmids present in all the *Shigella* and EIEC strains studied have homology with RepFIC. A replicon isolated from pWR110 turned out to resemble closely the replicon of the IncFII plasmid R1, another member of the RepFIIA family. Our observations extend our previous findings (2, 15, 16) on the evolutionary conservation of this replicon family.

MATERIALS AND METHODS

Bacterial strains. A total of 40 clinical isolates (29 *E. coli* and 11 *Shigella* strains) were used in this study. Their serogroups, resistance markers, and invasive ability, which was assayed by the Sereny test (21), are presented in Table 1. *E. coli* C600 (1), TB1 [Δ(*lac-pro*) *rpsL ara thi* φ80 *lacZ* M15 *hsdR*] (Bethesda Research Laboratories, Inc.), and JM107 (28) were used as hosts for the constructed miniplasmids, pUC subclones, and M13 bacteriophages (25), respectively.

Plasmid isolation and hybridization. Plasmid DNA was isolated as described by Kado and Liu (6) from 3-ml overnight cultures grown in tryptone-yeast extract medium. The DNA was precipitated, and all of it was applied to the gel. It was hybridized in 50% formamide–4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% sodium dodecyl sulfate–250 μg of denatured salmon sperm DNA per ml at 37°C and washed in 2× SSC four times at room temperature, as described previously (7). These conditions allow a calculated mismatch of about 20%. To assess the homology with RepFIB and RepFIC replicons, plasmids were hybridized to ^{32}P-labeled (12) replicon probes. The probe DNA was labeled by nick translation to a specific activity of about 5 ×

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TABLE 1. Clinical isolates used

Strain no.	Strain (serotype)	Source[a]	Antibiotic resistance	Sereny test
1	*E. coli* (O28ac) 3319-71	CDC	Sensitive	–
2	*E. coli* (O29) P1196-84	Mexico	Su^r Sm^r Tc^r Cm^r Km^r Ap^r	–
3	*E. coli* (O112ac) 1395-69	CDC	Ap^r	–
4	*E. coli* (O143) 1551-73	CDC	Ap^r	–
5	*E. coli* (O144) 4207-56	CDC	Tc^r Ap^r	–
6	*E. coli* (O28ac) 14267-84	EPM	Su^r	+
7	*E. coli* (O28ac) 100-82	EPM	Km^r Ap^r	+
8	*E. coli* (O28ac) 9-82::Tn*5*	EPM	Km^r Ap^r	+
9	*E. coli* (O29) 45-81	EPM	Su^r Sm^r Tc^r Cm^r	+
10	*E. coli* (O29) 79-82	Chile	Km^r	+
11	*E. coli* (O29) 127-82	EPM	Sensitive	+
12	*E. coli* (O124) 280-83	EPM	Su^r Sm^r Tc^r Ap^r	+
13	*E. coli* (O124) 5800-82	EPM	Su^r Sm^r Tc^r Ap^r	+
14	*E. coli* (O124) 27816-84	EPM	Su^r Sm^r Tc^r Ap^r	+
15	*E. coli* (O136) WMP-83	EPM	Su^r Sm^r Tc^r Km^r Ap^r	+
16	*E. coli* (O136) 223-83	EPM	Su^r Ap^r	+
17	*E. coli* (O136) 367-83	EPM	Su^r Tc^r Ap^r	+
18	*E. coli* (O143) 267-83	EPM	Su^r Tc^r Ap^r	+
19	*E. coli* (O143) 65-82	EPM	Sensitive	+
20	*E. coli* (O143) 122-83	Chile	Sensitive	+
21	*E. coli* (O144) 872-82	EPM	Su^r Tc^r	+
22	*E. coli* (O144) 6-84	EPM	Su^r	+
23	*E. coli* (O152) 27528-84	EPM	Su^r Sm^r Tc^r Km^r Ap^r	+
24	*E. coli* (O152) 26957-84	EPM	Su^r Tc^r Ap^r	+
25	*E. coli* (O152) 28211-84	EPM	Su^r Tc^r Ap^r	+
26	*E. coli* (O164) Saigon	EPM	Su^r	+
27	*E. coli* (O164) 24564-84	EPM	Su^r Sm^r Ap^r	+
28	*E. coli* (O167) 2383-83	EPM	Su^r Sm^r Tc^r Km^r Ap^r	+
29	*E. coli* (O167) 52-82	EPM	Su^r Sm^r	+
30	*S. dysenteriae* (A7) 378-83	EPM	Su^r Sm^r Tc^r Ap^r	+
31	*S. dysenteriae* (A1) 3044-74	CDC	Sensitive	+
32	*S. dysenteriae* (A5) 853-59	CDC	Sensitive	+
33	*S. flexneri* 212-83	EPM	Su^r Sm^r Tc^r Cm^r Ap^r	+
34	*S. flexneri* 219-83	EPM	Su^r Sm^r Tc^r Cm^r Ap^r	+
35	*S. flexneri* M90-T::Tn*5*	Sansonetti	Km^r	+
36	*S. boydii* 9-81	EPM	Su^r Sm^r Tc^r Cm^r	+
37	*S. boydii* (14) 227-81	Chile	Sensitive	+
38	*S. boydii* (5) 3408-67	CDC	Sensitive	+
39	*S. boydii* (3) 1052-50	CDC	Sensitive	+
40	*S. sonnei* 213-83	EPM	Ap^r	+

[a] Abbreviations: CDC, Centers for Disease Control, Atlanta, Ga.; EPM, Escola Paulista de Medicina, Sao Paulo, Brazil; Sansonetti, P. J. Sonsonetti, Pasteur Institute, Paris, France.

10^7 cpm/μg (7). To prepare each probe, fractionated fragments of the replicons were electroeluted onto DEAE membranes (NA-45; Schleicher & Schuell, Inc.) and extracted as recommended by the manufacturer.

Subcloning of RepINV and construction of RepINV miniplasmids. Restriction enzymes were purchased from New England BioLabs, Inc. Restricted and purified fragments were subcloned in linearized and alkaline phosphatase-treated pUC8, pUC18, or pUC19 and in M13mp11. To construct RepINV miniplasmids, we ligated RepINV-containing fragments to the omega fragment (11), consisting of a spectinomycin-streptomycin resistance gene flanked by transcriptional and translational terminators and synthetic polylinkers (*Eco*RI, *Sma*I, *Bam*HI, and *Hin*dIII).

Test for incompatibility. The incoming plasmid was transferred by transformation to the strain harboring the resident plasmid. This step was carried out with selection for the incoming plasmid only. The offspring were purified once by streaking to single colonies onto the same selective medium. They were then tested by replica plating for unselected markers present in the resident plasmid. As a control, we grew the recipient strain alone without selection for its plasmid and then measured spontaneous loss of the plasmid. Loss of the resident plasmid as a result of incompatibility was corrected for spontaneous loss.

DNA sequencing. The sequence of the putative *inc* RNA-coding region was determined by the enzymatic chain termination method of Sanger et al. (17). The dideoxy sequencing reactions were carried out as described by Biggin et al. (3) and Messing (8).

RESULTS

Plasmid profiles of the strains. High-molecular-weight plasmids of each strain were denoted A, B, and C according to their size, A being the largest (Table 2). Plasmid A was assumed to be the pINV in all Sereny test-positive strains. This assumption is based on our observation that more than 100 tested virulent *Shigella* and EIEC strains harbor a large plasmid (120 to 140 megadaltons) similar in size to the A plasmid. Loss of such a plasmid was always correlated with simultaneous loss of virulence (23, 24). Also, pWR110, the plasmid A present in strain no. 35, was previously shown to be a pINV (20). Strain no. 1 to strain no. 5 were included as virulence-negative controls, since they are Sereny test negative and do not belong to the invasive bioserotypes (22).

Hybridization of virulence-associated plasmids with RepFIC and RepFIB replicons. The probes used to assess the homology with the replicons of virulence-associated plasmids present in invasive strains were derived from the basic replicons RepFIB and RepFIC of the IncFI enterotoxin (Ent) plasmid, P307. They are located within 4.3- and 5.15-kbp *Eco*RI fragments, respectively (10). These fragments were subcloned in pBR325 (16; S. Saadi, unpublished data). The organization and partial restriction map of RepFIC are presented in Fig. 1a. For RepFIC, the 2.8-kbp *Sma*I-*Xho*I segment containing the replicon was used as a probe. The RepFIB probe comprised the 4.3-kbp *Eco*RI fragment mentioned above. The results obtained from the hybridization of RepFIC and RepFIB with various plasmids present in the clinical isolates are summarized in Table 2. All Sereny test-positive strains contain a pINV that has a region homologous with RepFIC but not with RepFIB. Some of these strains have additional high-molecular-weight plasmids that hybridize with RepFIC. They could be R plasmids, such as IncFII R plasmids, or they could be deletion derivatives of pINVs. Such deleted pINVs have been reported previously (20). Plasmid no. 20 seems to be of this type, since the strain carrying this plasmid is sensitive to all antibiotics tested (Table 1). Four of the five Sereny test-negative strains (Table 1, no. 1 to 5) which do not carry pINVs have plasmids that hybridize with both RepFIB and RepFIC or RepFIB only (Table 2). The fifth strain contains a plasmid A that hybridizes with RepFIC only. This may be an IncFII R plasmid. An example of hybridization with the replicon probes is shown in Fig. 1b and c.

Isolation of RepINV miniplasmids obtained from pWR110. Strain no. 35 is a *S. flexneri* 5 strain whose pINV is plasmid pWR110, a Tn*5* insertion mutant of pWR100 (20). This plasmid hybridizes with RepFIC only. To define the region of pWR110 which is homologous with RepFIC and contains the basic replicon, the plasmid was digested with *Eco*RI restriction endonuclease and fractionated fragments were hybridized with the RepFIC probes. A fragment of about 20 kbp was found to be homologous with RepFIC of P307 (10). This fragment was isolated and cloned in the *Eco*RI site of pUC8, the resultant plasmid being denoted pRMS1 (Fig. 2). The same fragment was ligated to the omega fragment of

TABLE 2. Hybridization of pINVs from different invasive strains with the RepFIB and RepFIC replicon probes

Strain no.[a]	Strain (serotype)	Presence of pINV	Hybridization with:					
			RepFIB HMW[b] plasmid			RepFIC HMW plasmid		
			A	B	C	A	B	C
1	*E. coli* (O28ac) 3319-71	−	+	−		+	−	
2	*E. coli* (O29) P1196-84	−	+			−		
3	*E. coli* (O112ac) 1395-69	−	+			+		
4	*E. coli* (O143) 1551-73	−	−			−		
5	*E. coli* (O144) 4207-56	−	−	−		+	+	
6	*E. coli* (O28ac) 14267-84	+	−	−		+	+	
7	*E. coli* (O28ac)100-82	+	−			+		
8	*E. coli* (O28ac) 9-82::Tn*5*	+	−			+		
9	*E. coli* (O29) 45-81	+	−	−		+	+	
10	*E. coli* (O29) 79-82	+	−			+		
11	*E. coli* (O29) 127-82	+	−			+		
12	*E. coli* (O124) 280-83	+	−	−	−	+	−	+
13	*E. coli* (O124) 5800-82	+	−	−		+	−	
14	*E. coli* (O124) 27816-84	+	−	−	−	+	−	−
15	*E. coli* (O136) WMP-83	+	−	−	−	+	+	+
16	*E. coli* (O136) 223-83	+	−	−		+	+	
17	*E. coli* (O136) 367-83	+	−	−		+	−	
18	*E. coli* (O143) 267-83	+	−	−	−	+	−	+
19	*E. coli* (O143) 65-82	+	−			+		
20	*E. coli* (O143) 122-83	+	−	−		+	+	
21	*E. coli* (O144) 872-82	+	−	−	−	+	−	+
22	*E. coli* (O144) 6-84	+	−	−		+	+	
23	*E. coli* (O152) 27528-84	+	−	−	−	+	−	+
24	*E. coli* (O152) 26957-84	+	−	−		+	+	
25	*E. coli* (O152) 28211-84	+	−	−	−	+	+	+
26	*E. coli* (O164) Saigon	+	−	−		+	+	
27	*E. coli* (O164) 24564-84	+	−	−		+	+	
28	*E. coli* (O167) 2383-83	+	−	−	−	+	+	+
29	*E. coli* (O167) 52-82	+	−	−		+	−	
30	*S. dysenteriae* (A7) 378-83	+	−	−		+	−	
31	*S. dysenteriae* (A1) 3044-74	+	−			+		
32	*S. dysenteriae* (A5) 853-59	+	−			+		
33	*S. flexneri* 212-83	+	−			+		
34	*S. flexneri* 212-83	+	−	−		+	+	
35	*S. flexneri* M90-T::Tn*5*	+	−			+		
36	*S. boydii* 9-81	+	−			+		
37	*S. boydii* (14) 227-81	+	−			+		
38	*S. boydii* (5) 3408-67	+	−			+		
39	*S. boydii* (3) 1052-50	+	−			+		

[a] The strain numbers in this table correspond to those in Table 1.
[b] HMW, High molecular weight.

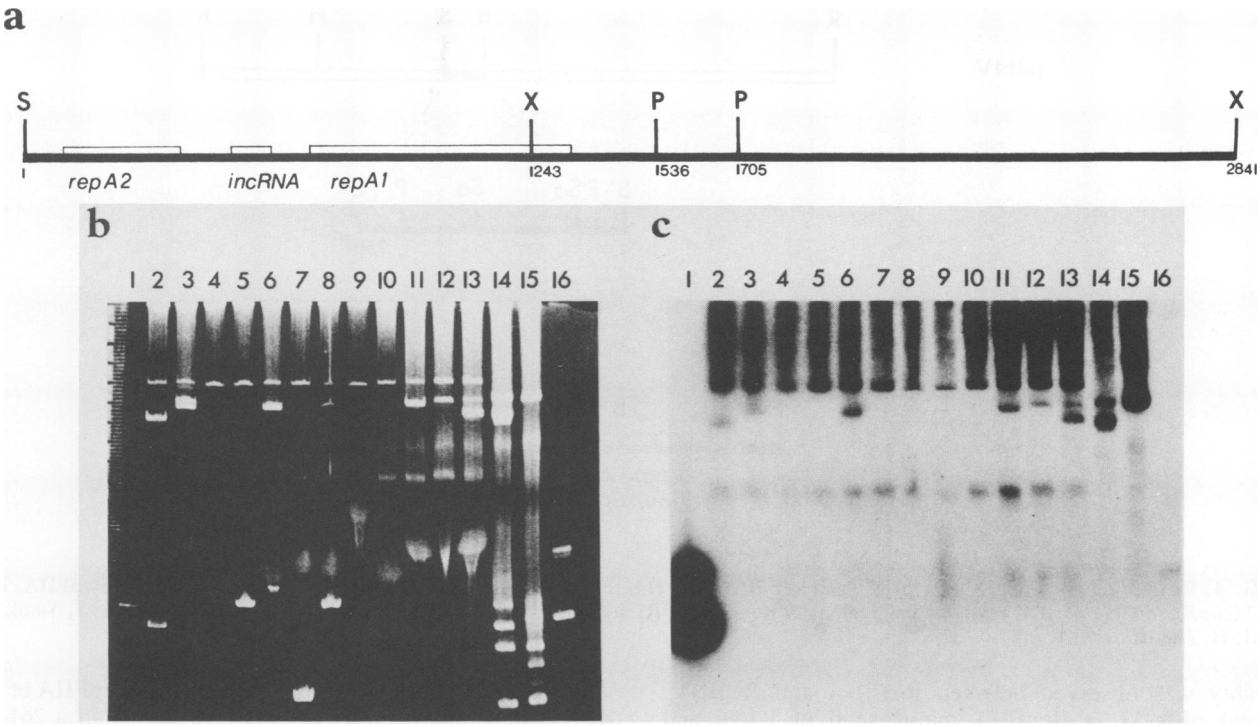

FIG. 1. (a) Restriction endonuclease map of RepFIC of P307 (16). The *repA2*, *inc* RNA, and *repA1* coding regions are shown. Abbreviations: S, *Sma*I; X, *Xho*I; P, *Pst*I. (b) Electrophoresis in a 0.7% agarose gel of plasmid DNA isolated as described by Kado and Liu (6). Lanes: 1 and 16, RepFIC and RepFIB, controls for hybridization; 2 to 8 and 13, EIEC Sereny test-positive strains corresponding to no. 13, 14, 17, 8, 9, 10, 29, and 15, respectively, in Tables 1 and 2; 9 to 12, *Shigella* Sereny test-positive strains corresponding to no. 31, 32, 34, and 30, respectively, in Tables 1 and 2; 14, EIEC Sereny test-negative and pINV$^-$ strain (no. 5 in Tables 1 and 2); 15, an enteropathogenic *E. coli* strain. (c) Autoradiograph of the gel in panel b after hybridization with the RepFIC probe. It should be noted that with large plasmids it is difficult to avoid some linearization, as shown by the smears up to the hybridized plasmid band and by the faint hybridization of the chromosomal band as a result of entrapped linear plasmid DNA. This is not seen in the controls in lanes 1 and 16.

pHP45Ω (11) to construct pRMS2. Plasmid pRMS2 was digested with *Bam*HI and *Bgl*II restriction endonucleases, giving rise to fragments of about 14 and 6 kbp. The 6-kbp fragment was shown to be homologous with RepFIC and was ligated to the omega fragment digested with *Bam*HI to construct pRMS3. Since pRMS2 and pRMS3 can replicate autonomously, these results confirm that the region of pWR110 which is homologous with RepFIC carries a functional replicon.

Incompatibility of RepINV plasmids. Our hybridization studies indicated that RepINV of pWR110 is homologous with RepFIC. The latter resembles RepFIIA of R1 (9, 14) (Fig. 3). It can be seen that both replicons possess sites for *Bgl*II, *Pst*I, *Sau*3A, and *Sma*I in a 780-bp DNA region. It is known that replication of RepFIIA of R1 and R100 and of RepFIC of P307 is regulated by a countertranscript RNA which also determines incompatibility (9, 16, 27).

Because of the similarity between RepINV, RepFIIA, and RepFIC it was of interest to determine the incompatibility behavior of pWR110 and its derivatives. This was assessed by their ability to coexist with plasmids carrying RepFIC or RepFIIA or both. RepFIC and RepFIIA replicons are compatible (16). Furthermore, RepFIIAs of R1 and R100 show identical incompatibility behavior and thus can be interchanged in incompatibility tests. Weak incompatibility was observed between RepFICs of P307 and RepINV (Table 3): plasmid pWM113 (a RepFIC subclone) inhibits replication of pRMS3 (a RepINV miniplasmid) by 15%, and plasmid pRMS1 (a RepINV subclone) inhibits replication of pSS3945 (a RepFIC miniplasmid) by 35% (lines 1 and 2). No incom-

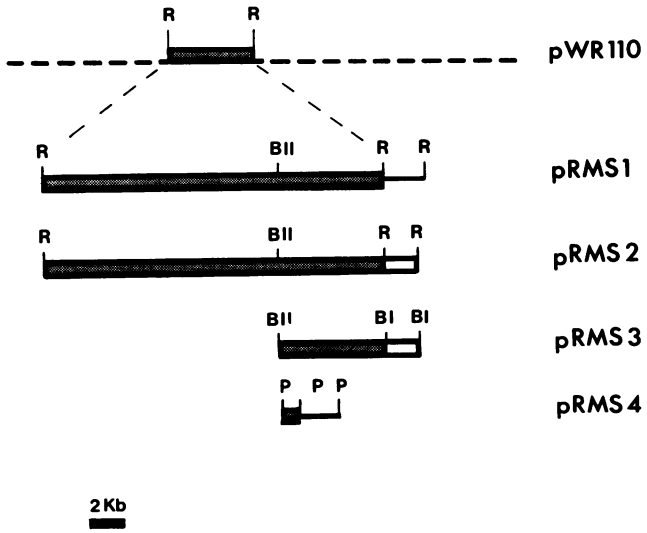

FIG. 2. Construction of pRMS1, pRMS2, pRMS3, and pRMS4. pWR110 was digested with *Eco*RI, and the 20-kbp fragment was cloned in pUC8 to construct pRMS1. The same fragment was ligated to the omega fragment from pHP45Ω, giving rise to pRMS2. pRMS2 was digested with *Bam*HI and *Bgl*II, and the 6-kbp fragment was ligated to omega, giving rise to pRMS3. The 500-bp *Pst*I fragment of pRMS3 was cloned in pUC19 to construct pRMS4. Abbreviations: R, *Eco*RI; BI, *Bam*HI; BII, *Bgl*II; P, *Pst*I.

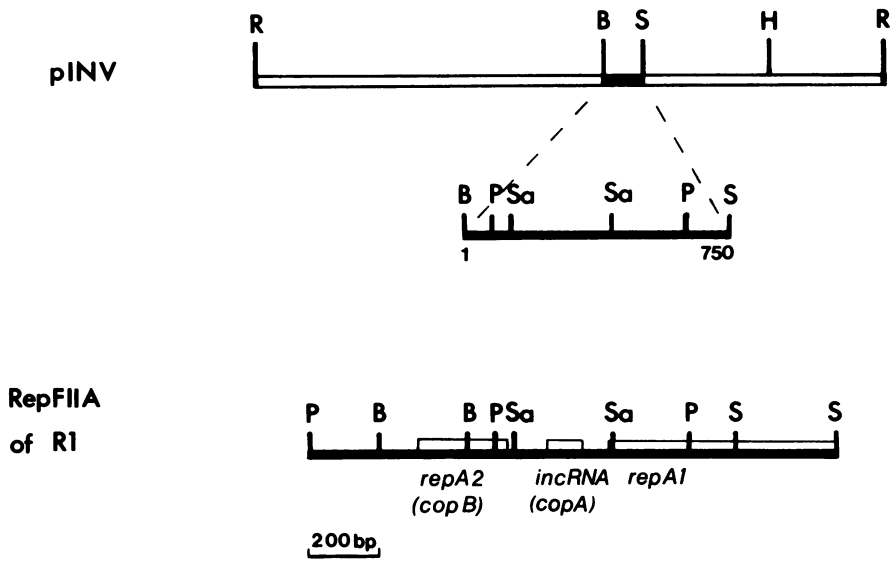

FIG. 3. Restriction maps of pINV (RepINV) and RepFIIA of R1 (14). For comparison, the pINV map is aligned with that of RepFIIA. The *repA2* (*copB*), *inc* (*copA*), and *repA1* coding regions of RepFIIA of R1 are shown. Abbreviations: P, *Pst*I; B, *Bgl*II; Sa, *Sau*3A; S, *Sma*I; R, *Eco*RI; H, *Hin*dIII.

patibility was observed between RepFIIA and RepINV: plasmid pRR933, a RepFIIA miniplasmid of R100, and pRMS3 were able to coexist (lines 3 and 4). It is unlikely that the absence of incompatibility between pRR933 and pRMS3 is due to their lower copy number, since we have found that low-copy-number IncFII miniplasmids always exhibit the same incompatibility toward a similar resident replicon as do the corresponding higher-copy-number subclones (unpublished experiments).

The *inc* gene of RepFIIA of R1 is located within a 550-bp *Pst*I fragment. This fragment, which is also present in RepINV (Fig. 3), was assumed to contain the incompatibility determinant of this basic replicon. Thus, the 550-bp *Pst*I fragment was cloned into the *Pst*I site of pUC19 to construct pRSM4 (Fig. 2). pRMS4 was able to inhibit replication of pWR110 and pRMS3 (Table 3), indicating that an incompatibility determinant of pINV resides within the 550-bp *Pst*I fragment.

Determination of the nucleotide sequence of the RepINV-*inc* RNA-coding region. Compatibility between related replicons is not uncommon. We reported that RepFIC of P307 is compatible with RepFIIA, owing to a few base pair substitutions in the *inc* RNA-coding region (16). This has also been shown for the Rep1 of ColV2-K94, which is similar to RepFIIA of R1 but is compatible (26). The incompatibility behavior of RepINV toward RepFIC and RepFIIA prompted us to determine the nucleotide sequence of the RepINV-*inc* RNA-coding region.

Examination of the nucleotide sequence of RepFIIA of R1 reveals that the *inc* RNA gene is located within a 261-bp *Sau*3A fragment which is also present in RepINV. This *Sau*3A fragment was excised from pRMS4 and inserted into the *Bam*HI site of M13mp11 to construct mpSSM5 and mpSSM6. The sequence of this region was determined from both strands and was compared with the sequences of RepFIC of P307, RepFIIA of R1, and Rep1 of ColV2-K94 (26). The results are shown in Fig. 4. The region reveals 20 mismatches with P307, 10 mismatches with R1, and 14 mismatches with ColV2-K94, in a 261-bp region.

It can be seen (Fig. 4) that most of the base pair substitutions occur within the putative *inc* RNA-coding region (82 to 172), whereas the putative −10 (178 to 163) and −35 (201 to 206) RNA polymerase recognition sites of the *repA1* mRNA are highly conserved. The *inc* RNA has the potential to form two stem-loop structures. The 6 bases of the loop of the major hairpin are believed to be the major site of interaction of the *inc* RNA and its target, the leader transcript of the *repA1* mRNA. This interaction subsequently inhibits the translation of the *repA1* protein (9) and is believed to be responsible for the incompatibility of these replicons. We demonstrated (16) that although the *inc* RNA of RepFIC possesses identical bases in the loop of the major hairpin, it is compatible with RepFIIA replicons. Similarly, the putative *inc* RNA encoded by RepINV possesses an identical loop (Fig. 4, the box below the sequence), but owing to base pair substitutions in the stem of the major hairpin, which

TABLE 3. Incompatibility tests performed with RepINV[a]

Incoming plasmid	Incoming replicon	Resident plasmid	Resident replicon	% Loss of resident plasmid	% Loss of control	Conclusion
pWM113	RepFIC/ColE1	pRMS3	RepINV	15	0	Weakly incompatible
pRMS1	RepINV/ColE1	pSS3945	RepFIC	35	17.5	Weakly incompatible
pRR933	RepFIIA	pRMS3	RepINV	0	0	Compatible
pRMS3	RepINV	pRR933	RepFIIA	0	0	Compatible
pRMS4	RepINV-*inc* RNA	pWR110	RepINV	100	0	Incompatible
pRMS4	RepINV-*inc* RNA	pRMS3	RepINV	100	0	Incompatible

[a] pWM113 and pSS394 are subclone and miniplasmid of RepFIC, respectively (16). pRR933 is a miniplasmid and pDXRR3 is an *inc* RNA subclone of R100 (26).

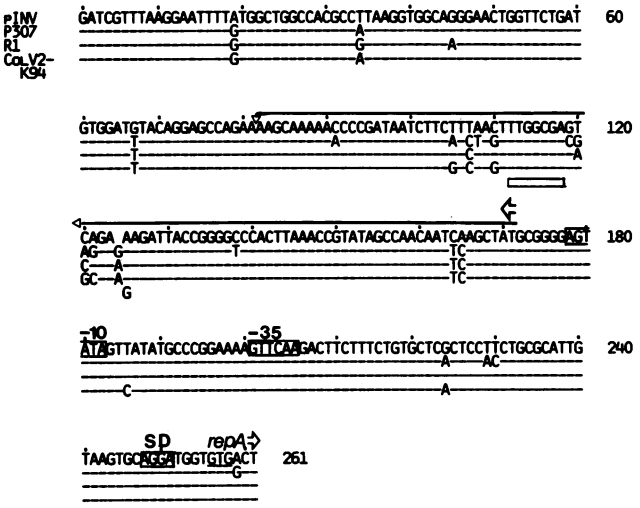

FIG. 4. Comparison of RepINV of pINV, RepFIC of P307, RepA of R100, and ColV2-K94 *inc* RNA-coding sequences and their flanking regions. Numbers to the right of the sequence correspond to the RepINV sequence. The arrow above the sequence indicates the location and the direction of *inc* RNA. The box below the sequence shows the location of the loop of the major hairpin structure in this RNA. The −35 and −10 RNA polymerase recognition sites which are identical to those suggested for P307 (16), R100 (14), and ColV2-K94 (26) are indicated. The Shine-Dalgarno sequence (SD) and the first amino acid of RepA1 protein are also marked. Dashes denote identical bases. A base deletion in pINV is indicated by a gap, and a single-base insertion in ColV2-K94 is indicated below the sequence.

apparently change the target-inhibitor complementarity, RepINV is only weakly incompatible with RepFIC of P307 and is compatible with RepFIIA of R100 (Table 3).

DISCUSSION

We have shown that a virulence plasmid (pINV) present in 35 invasive strains, including examples from all *Shigella* species and most of the EIEC serogroups, has homology with RepFIC, a basic replicon present in IncFI plasmids. No homology was observed with RepFIB, another basic replicon commonly found in IncFI plasmids. There is a third basic replicon, RepFIA (13), in many IncFI plasmids, and we are planning to test our 35 strains for hybridization with a RepFIA probe and 13 other replicon probes that have recently become available (P. L. Bergquist, M. Couturier, and W. K. Maas, unpublished experiments).

It seems likely that the replicon having homology with RepFIC is the only replicon present in pINVs. Besides the data with the probes, there are two findings that provide evidence for this statement. The observed two-way incompatibility of pWR110 with pRMS4 (Table 3) indicates that only one replicon is present in this plasmid, since a second replicon would rescue pWR110 when it was used as the resident plasmid in incompatibility tests. We have found that such rescue is responsible for the observed one-way incompatibility between plasmid pCG86, which contains two replicons (RepFIIA/FIC and RepFIB [10]), and R1, which contains one replicon (RepFIIA) (Maas, unpublished experiments). The other indication that there is only one replicon in pINVs was our inability to obtain self-replicating clones that did not hybridize with the RepFIC probe.

Replicons having homology with RepFIC are the most frequent replicons among plasmids belonging to IncF incompatibility groups but are not present in plasmids belonging to most other incompatibility groups (2). These replicons have been combined in a replicon family, the RepFIIA family (16). It is clear that the replicons present in pINVs belongs to the RepFIIA family and that pINVs are therefore evolutionarily related to group IncF plasmids. It is also interesting from the point of view of evolution that the same type of replicon appears to be present in all pINVs. This is in contrast to R plasmids, for which homologous resistance determinants are found to be associated with a variety of replicons, presumably owing to the importance of transposition in the dissemination of resistance genes.

We have shown that three members of the RepFIIA family, RepFIC of P307, RepFIIA of R1, and RepFIIA of R100, have three regions of homology, interspersed by two regions of nonhomology (16). Although RepINV was identified by its hybridization with RepFIC of P307, its restriction endonuclease map shows greater similarity with RepFIIA of R1 (Fig. 3). The sequence of one strand of a *Bgl*II-*Sau*3A fragment of RepINV which is also present in R1 has been determined (S. Saadi and R. M. Silva, unpublished data). This segment contains the carboxy-terminal part of *repA2* (i.e., *copB*), a gene for a putative repressor protein. This region is divergent among the three sequenced replicons of the RepFIIA family (14, 16), leading to three different *repA2* polypeptides. RepA2 of RepFIC in P307, RepFIIA in R100, and RepFIIA in R1 possess different carboxy-terminal regions but share 11 amino acids at their amino-terminal domain. Our sequence analysis indicates that the *repA2* sequence of RepINV is more closely related to the corresponding R1 sequence (about 99% homology) than to those of P307 and R100 (about 46% homology).

The 261-bp *Sau*3A fragment in RepINV contains the entire *inc* RNA-coding region and the initiation codon of a putative *repA1* protein. The comparison of this region in pINV with P307, R100, and ColV2-K94 is shown in Fig.4. The sequence of one strand of the *Sau*3A-*Pst*I fragment to the right of the 261-bp *Sau*3A fragment extending into the *repA1* gene in pINV (Fig. 3) has also been determined (Saadi and Silva, unpublished data) and shows about 84% homology with R1 and R100. The replicon in R1 and R100 is not homologous with RepFIC in this region, and, as expected, neither is RepINV.

In view of these findings, it is apparent that the nature of the probes used is crucial in determining relationships of RepINV with other replicons. The RepFIC probe we used (Fig. 1) contains regions of homology and regions of nonhomology with RepFIIA. By using shorter probes derived from different regions of the RepFIC replicon, we could have discerned the relationship between RepFIC and RepINV more precisely.

Finally, the replicon probing we have described here has value for the detection of pINVs in clinical isolates, since the only other group of plasmids that seem to contain one replicon of the RepFIIA family are IncFII R plasmids. Hybridization with RepFIC only was not observed for plasmids of several other clinical isolates, including classical enteropathogenic *E. coli* strains and *Salmonella* species (R. M. Silva, unpublished data). Thus, a positive test with a RepFIC probe only is indicative of a pINV plasmid, especially if the strain harboring the pINV is sensitive in tests for antibiotics.

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