Distribution of the *Escherichia coli* K12 Insertion Sequences IS1, IS2 and IS3 Among Other Bacterial Species

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A total of 15 bacterial species were screened for the presence of the *Escherichia coli* K12 insertion sequences IS1, IS2 and IS3 by Southern blotting and DNA hybridization. The only positive signals obtained were for IS1 in *Serratia marcescens*, where the sequence was present on a plasmid, and a weak hybridization to IS3 in *Erwinia carotovora*. This shows that the capacity for transposition alone is not sufficient to allow the spread of sequences through a wide range of bacterial species.

INTRODUCTION

The DNA insertion sequences IS1, IS2 and IS3 are natural constituents of the *Escherichia coli* K12 chromosome, where they are present in multiple copies (Saedler & Heiss, 1973; Deonier *et al.*, 1979). They also occur on various *E. coli* plasmids (Hu *et al.*, 1975) and phages (Iida *et al.*, 1978). They can transpose to new sites and account for a significant proportion of spontaneous mutations (for reviews see Starlinger & Saedler, 1976; Starlinger, 1980).

In view of their transposition properties and occurrence on plasmids it is theoretically possible for insertion sequences from *E. coli* K12 to become established in a variety of bacterial species. Thus the study of the distribution of insertion sequences among bacterial species, and their sequence divergence, should give valuable information about rates of evolution, and the frequency of genetic exchange between species. This is especially interesting because the selective pressures on transposable elements that do not code for any known essential function in the host cell should be very different from those acting on other DNA sequences.

We therefore decided to search a representative range of bacterial species by using DNA hybridization specific for IS1, IS2 and IS3. The species were chosen as representatives of groups commonly studied in the laboratory, and also for their low pathogenicity, with especial emphasis on soil bacteria.

METHODS

Bacterial strains. The bacterial strains are shown in Table 1; *E. coli* K12 strain 431 carries a deletion from *nadA* through *gal* to *pgl*.

Bacteria were grown on PH1 medium, containing (per litre): 10 g peptone (Difco), 1 g yeast extract (Difco), 4 g glucose; pH 7.0. Exponentially growing cultures (shaking at 33 °C) of between 100 ml and 1 litre in volume were used for isolation of DNA. The cells were lysed with lysozyme and detergent and extracted with chloroform/isoamyl alcohol (24:1, v/v) as described by Marmur (1961). The lysate was then further purified by CsCl-ethidium bromide equilibrium centrifugation as described by Ghosal & Saedler (1977). The resulting DNA preparations were of high molecular weight (at least 20 kb as estimated by agarose gel electrophoresis).

Plasmids from *E. coli* K12 strain 431 and the *Serratia marcescens* plasmid(s) were purified as described by Ghosal & Saedler (1977).

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Table 1. *Bacterial strains*

Bacterium	Strain no.*
<i>Bacillus subtilis</i>	5029
<i>Clostridium rubrum</i>	DSM 53
<i>Corynebacterium poinsettiae</i>	DSM 20149
<i>Dactylosporangium aurantiacum</i>	DSM 43157
<i>Erwinia carotovora</i>	DSM 30168
<i>Escherichia coli</i> K12	431
<i>Geodermatophilus obscurus</i> var. <i>dictyosporus</i>	DSM 43161
<i>Lactobacillus plantarum</i> subsp. <i>plantarum</i>	DSM 20174
<i>Myxococcus fulvus</i>	DSM 434
<i>Proteus vulgaris</i>	5031
<i>Pseudomonas aeruginosa</i>	5028
<i>Pseudomonas cepacia</i>	DSM 50181
<i>Rhizobium japonicum</i>	5030
<i>Serratia marcescens</i>	5027
<i>Streptomyces niveus</i>	5032
<i>Xanthomonas campestris</i>	DSM 50051

* Strain 431 was from the laboratory collection (Ghosal & Saedler, 1977). Strains 5027–5032 were kindly donated by Dr J. Weckesser. The strains with a DSM prefix were from the Deutsche Sammlung von Mikroorganismen.

Hybridization probes for IS1, IS2 and IS3. Two different probes were used for IS1.

(1) Plasmid pDG128/9 Δgal (Sommer *et al.*, 1981). This was formed by two successive deletions, in opposite directions, from the IS1 in *galOP-128::IS1* carried on the plasmid pDG1 (Ghosal & Saedler, 1977). The first deletion removes *galOP*, *chlD* and *pgl* and leaves only about 700 bp between the end of IS1 and the *EcoRI* site. The deletion to the other side has removed the *gal* operon, the lambda sequences and the *EcoRI* site. Thus, the plasmid consists of IS1, most of pSC101 and about 700 bp of *E. coli* DNA.

(2) A purified 1.2 kb *PstI*–*EcoRI* fragment from the plasmid pJR50512 (Reif & Arber, 1981) that carries 580 bp of IS1 from the *PstI* site and part of the chloramphenicol acetyl transferase gene from Tn9 (it is actually the larger *PstI*–*EcoRI* internal fragment from Tn9: Alton & Vapnek, 1979).

Two probes were also used for IS2.

(1) The 720 bp internal *HincII*–*HindIII* fragment of IS2 from the plasmid pDG12 (Ghosal *et al.*, 1979).

(2) A fragment from the plasmid pDG128/10R1 (Sommer *et al.*, 1981). This is a derivative of pDG1 (Ghosal & Saedler, 1977) which carries the IS1 insertion *galOP-128::IS1* (sequenced by Kühn *et al.*, 1979). An IS2 had inserted (in orientation II) in IS1 19 bp from the *galE* end of IS1 (unpublished results). The actual probe used was a 1.35 kb *HindIII* fragment which consisted of 871 bp of IS2 (Ghosal *et al.*, 1979), 19 bp of IS1 and about 450 bp of *galE* (Ghosal & Saedler, 1977).

The IS3 probe was the 1.05 kb internal *HindIII* fragment from the plasmid pPPP4-IS2-437::IS3 (Sommer *et al.*, 1979).

Restriction digests, gel electrophoresis, fragment purification, Southern transfers and hybridization. Restriction digests, agarose gel electrophoresis and purification of restriction fragments from gels were as described by Ghosal & Saedler (1977). To obtain complete digestion of total bacterial DNA 5–10 units of *EcoRI* ($\mu\text{g DNA}$)⁻¹ were used in overnight digestions. All restriction enzymes were bought from Boehringer except *HincII* (New England Biolabs).

Transfer of DNA from agarose gels to nitrocellulose (Southern, 1975), ³²P-labelling of probes by nick-translation, hybridization and autoradiography were as described by Wienand & Feix (1980).

RESULTS

Hybridization with total DNA

DNA from the species shown in Table 1 was digested with the restriction enzyme *EcoRI* and the resulting fragments were separated by agarose gel electrophoresis. As expected, there were many bands and the positions of bands differed for the different species. The DNA from such gels was transferred to nitrocellulose filters and hybridized with ³²P-labelled DNA containing IS1, IS2 or IS3.

When an IS1-containing probe was used, four bands of hybridization were seen with DNA from *E. coli* K12 and the only other species showing strong hybridization was *Serratia marcescens*.

Fig. 1. DNA (5 µg) from various species was digested with *Eco*RI and separated by electrophoresis on a 1% agarose gel. After transfer to a nitrocellulose filter, the DNA was hybridized with a 32 P-labelled DNA fragment that carried part of IS1 and part of a chloramphenicol acetyltransferase gene (see Methods). The autoradiograph from this experiment is shown. DNA from the following species was used: track 1, *S. marcescens*; track 2, *P. aeruginosa*; track 3, *B. subtilis*; track 4, *R. japonicum*; track 5, *P. vulgaris*; track 6, *S. niveus*; and track 7, *E. coli* K12. Details of strains are given in Table 1.

cens (Fig. 1). Both of the IS1 probes used (see Methods) gave the same pattern of hybridization, and control experiments (data not shown) showed that they did not cross-hybridize (except for the IS1 regions) so it seemed likely that the sequence identified in *S. marcescens* was due to IS1. Occasionally, faint bands were seen with other species, but they were not reproducible and were not characterized further.

Both IS2 probes gave similar hybridization patterns. [The *galE* fragment on one of the probes (see Methods) did not give additional hybridization with *E. coli* K12 DNA because strain 431 is deleted for the *gal* operon.] There were about seven bands with *E. coli* K12 and no significant hybridization with the other species (data not shown). Sometimes faint bands were seen but these were not reproducible.

The IS3 probe gave six to eight bands with *E. coli* K12 DNA. It also gave a weak band of size 2 kb with *Erwinia carotovora* DNA (Fig. 2).

Plasmid DNA from Serratia marcescens

The IS1 band in *S. marcescens* DNA was very intense (Fig. 1), suggesting that it was present at a higher copy number than single-copy chromosomal sequences. We therefore purified plasmid DNA from this strain. A covalently closed circular (CCC) DNA band was seen in CsCl-ethidium bromide gradients of DNA from this strain. When this fraction was further separated by agarose gel electrophoresis, three bands were seen, corresponding to CCC DNA of about 10 kb in one case and larger than 20 kb in the other two cases (data not shown). The plasmid DNA gave one band of hybridization the same size as that seen in total DNA, so the IS1 sequence seems to be plasmid-borne. The plasmid DNA was digested with a variety of restriction enzymes, transferred to nitrocellulose and hybridized with an IS1 probe. In all cases except for *Hinc*II, only one band was seen (Fig. 3). With *Hinc*II, two to four bands were seen, depending on the amount of enzyme added. The two bands of about equal intensity (Fig. 3), which were always present, could be due to partial digestion, as we have had problems in obtaining complete digestion with this enzyme in other plasmid preparations. Alternatively, there may be two

Fig. 2. Southern blottings of total bacterial DNA (5 µg samples, *Eco*RI digested as in Fig. 1) were hybridized with a 32 P-labelled IS3 probe (see Methods). DNA from the following species was used: track 1, *E. coli* K12; track 2, *C. poinsettiae*; track 3, *P. cepacia*; track 4, *L. plantarum*; and track 5, *E. carotovora*. Details of strains are given in Table 1.

Fig. 3. Samples (1 µg) of DNA from the plasmid fraction of *S. marcescens* were digested with various restriction enzymes and hybridized with an IS1 probe after Southern blotting (as described in Fig. 1). The following enzymes were used: track 1, *Eco*RI; track 2, *Hind*III; track 3, *Hinc*II; track 4, *Bam*HI; track 5, *Pst*I; track 6, *Bam*HI + *Pst*I (double digest); and track 7, *Hind*III + *Pst*I (double digest).

copies of IS1 on the plasmid (although this would be difficult to reconcile with the size of the bands seen with the other enzymes and enzyme combinations). Finally, the IS1 copy in *S. marcescens*, unlike other known IS1 copies (Ohtsubo & Ohtsubo, 1978), might contain a *HincII* site. Sequence divergence has been observed for other copies of IS1; Cornelis & Saedler (1980) reported that an IS1 derived from a *Yersinia enterocolitica* plasmid lacked a *PstI* site.

DISCUSSION

Restriction endonuclease-digested DNA of *E. coli* K12 strain 431 gave four, seven, and six to eight bands that hybridized with probes for IS1, IS2 and IS3, respectively. We made no attempt to measure accurately the molecular weights of these bands, and our gel system did not resolve bands bigger than about 10 kb very well. Within these limitations, the results were consistent with published data for IS1 (Nyman *et al.*, 1981) and IS3 (Deonier *et al.*, 1979), and the number of IS2 bands was compatible with the five copies estimated by Saedler & Heiss (1973) by quantitative hybridization.

In agreement with Nisen *et al.* (1979) and Nyman *et al.* (1981), we found no copies of IS1 in *Pseudomonas aeruginosa* and *Bacillus subtilis*. We also found IS1 absent in 12 other species. Nisen *et al.* (1979) found no IS1-specific hybridization in *S. marcescens*, whereas Nyman *et al.* (1981) found two bands. We found a very strongly hybridizing band that proved to be carried on a plasmid. It seems likely that this was due to IS1-specific sequences because: (1) the same size of band was obtained with two different probes that did not cross-hybridize (except for the IS1 sequences); and (2) in one case, the probe only contained part of a chloramphenicol acetyl transferase gene apart from IS1 sequences (see Methods) and our *S. marcescens* strain was sensitive to 10 µg chloramphenicol ml⁻¹. Therefore, the differences observed probably reflect differences in plasmid complement between the strains used by different groups.

We found no hybridization with our IS2 probe to any species other than *E. coli*. Nisen *et al.* (1979) found weak hybridization to the DNA of *S. marcescens*, *P. aeruginosa* and *B. subtilis*. This is not inconsistent with our results, as we used more stringent hybridization conditions because we were only interested in identifying sequences showing substantial homology to IS sequences. Thus, Nisen *et al.* (1979) found cross-hybridization between IS1 and IS2 (which only share four imperfectly matching sequences, each about 20 bp long) whereas our control experiments (data not shown) showed no hybridization between IS1 and IS2.

In the case of IS3, we observed hybridization with DNA from *E. carotovora*. This band was weak and we did not test whether the homology was chromosomal or plasmid-borne. In all other cases reproducible hybridization was not observed.

We tested 15 species in addition to *E. coli* for sequences homologous to IS1, IS2 and IS3. We only obtained two positive responses, and these were in the species *E. carotovora* and *S. marcescens*, which of the species shown in Table 1 are normally considered the most closely related to *E. coli* (see *Bergey's Manual of Determinative Bacteriology*: Buchanan & Gibbons, 1974). IS2 seems to have a very narrow host range, not being present in any of the species we tested and also being absent from *Salmonella typhimurium* (Nisen *et al.*, 1979). IS1 is present in species closely related to *E. coli* (Nisen *et al.*, 1979; Nyman *et al.*, 1981) although these two groups of workers obtained different results from each other for *E. coli* W and *S. typhimurium*. This difference observed between IS1 and IS2 supports earlier work by Rak (quoted in Starlinger & Saedler, 1976). Thus, despite their ability to transpose, the *E. coli* K12 insertion sequences IS1, IS2 and IS3 are not widely spread among other bacterial species. This might reflect a low frequency of genetic exchange between distantly related species of bacteria.

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