Biochemical Analysis of Spontaneous fepA Mutants of Escherichia coli

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(Received 22 June 1987; revised 17 November 1987)

The fepA gene of Escherichia coli encodes the outer-membrane receptor protein for ferrienterobactin. Previous genetic studies indicated that fepA mutations occur frequently and suggested that most of the mutations were deletions. In this work seven spontaneous fepA mutations were analysed by enzyme assay (enterobactin synthase and enterobactin esterase) and by DNA hybridization studies. In two strains, UT500 and UT700, the mutations were confined to the fepA gene. In the remaining mutants, the mutations were large deletions; in several cases, 27 kb or more of DNA had been lost. The deletions, all of which eliminated approximately the left half of the enterobactin gene cluster, extended from the vicinity of the fepC gene counterclockwise into the chromosome. A minimum of three clockwise endpoints were identified and at least two counterclockwise endpoints were detected. The variation in endpoints among the deletions argues against the involvement of a normal transposon in their formation. Also, unexpected homology was found between enterobactin gene cluster DNA and lacPOZ and pSC101.

INTRODUCTION

The endogenous high-affinity iron-transport system of Escherichia coli uses the siderophore enterobactin (enterochelin), which is a cyclic trimer of 2,3-dihydroxy-N-benzoyl-L-serine (DBS). For this uptake system to operate, the products of at least eleven genes are specifically required. These genes occur in the order entD fepA fes entF fepC fepB entEBG(CA) (Coderre & Earhart, 1984; Fleming et al., 1985; Pierce & Earhart, 1986) and are located in a cluster between 13.3 and 13.8 min on the chromosome (Bachmann, 1983). Seven genes (entA–G) are involved in enterobactin biosynthesis, three (fepA–C) code for transport proteins, and the fes product is necessary for the release of iron from the siderophore.

The fepA gene specifies the outer-membrane receptor protein for ferrienterobactin and colicins B and D. McIntosh et al. (1979) isolated a collection of spontaneous mutants lacking fepA by selecting and screening colicin-B-insensitive mutants. These mutants have been used in studies on thermoregulation of porin production (Lundrigan & Earhart, 1984), identification of the structural gene for outer-membrane protein OmpT (Rupprecht et al., 1983), endoprotease cleavage of FepA (Fiss et al., 1979) and T7 RNA polymerase (J. Grodberg, personal communication) and to identify an alternative iron-transport pathway (Pickett & Earhart, 1981), but they have not been systematically characterized. Most of the mutations appeared to be deletions, as no true revertants were isolated and many of the mutants showed greatly enhanced contransduction frequencies between purE (12.2 min) and fepA. It was suggested that the prevalence of deletions may have resulted from the presence of an insertion element, possibly the IS3 known to be in this region (Hu et al., 1975). Pugsley & Schnaitman (1978) also reported evidence for deletions in this region and noted an insertion sequence (IS) in the

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Abbreviations: DBS, 2,3-dihydroxy-N-benzoyl-L-serine; IS, insertion sequence.
Fig. 1. Enterobactin gene cluster. (a) Partial restriction map of the enterobactin gene cluster (E, EcoRI; H, HindIII; V, EcoRV). Portions of this region carried on several plasmids are also shown. (b) Physical map of some deletions extending into the enterobactin gene cluster as determined by this work.

vicinity. More recently, firm precedents for the appearance of insertion sequences near the genes for iron-transport systems were established when (i) several groups (McDougall & Neilands, 1984; Perez-Casal & Crosa, 1984; Lawlor & Payne, 1984) reported that the aerobactin-mediated iron-transport system on the ColV plasmid is flanked by IS1 elements and, (ii), that IS2 is also associated with the chromosomal aerobactin genes in some Shigella species (Lawlor & Payne, 1984).

As part of our studies on the enterobactin gene cluster and flanking regions of the chromosome, we have characterized here the extent of several fepA deletion mutations by enzyme assays and hybridization experiments. The idea that an IS was associated with this gene cluster and was responsible for the prevalence of deletion mutations was tested as well. A variety of right- and left-hand endpoints was detected among these deletions, which argues against the involvement of a normal insertion element in deletion formation. Unexpected homology was detected between DNA of the enterobactin cluster and plasmid pSC101 and lac DNA.

METHODS

Bacteria, plasmids and bacteriophage. The bacterial strains and plasmids used are listed in Table 1. Additional information regarding relationships among some of these plasmids and the genes in the 12–13:8 min region of the E. coli chromosome is given in Fig. 1. The phage λplacS7–MS505 (lacZ : : IS3) (Malamy et al., 1972) was supplied by M. H. Malamy.

Media and reagents. Cells were routinely grown in L broth (Earhart et al., 1979). MLT broth (Malamy et al., 1972) was used for growth of E. coli RV lysogenized with λplacS7–MS505 and, for extracts for enzyme assays, cells were grown in iron-depleted M9 medium (McIntosh & Earhart, 1977) with appropriate supplements as before (Pierce et al., 1983). Ferrienterobactin was isolated as described by Pierce et al. (1983). [32P]dCTP was purchased from New England Nuclear and ICN Pharmaceuticals, and [U-14C]serine from ICN Pharmaceuticals. Restriction enzymes were from New England Biolabs and Bethesda Research Laboratories.

DNA isolation. Large plasmids were isolated by either the procedure of Katz et al. (1973) or that of Hanson & Olsen (1978). The methods of either Birnboim & Doly (1979) or Holmes & Quiqley (1981) were used to isolate small plasmids. Chromosomal DNA and λ DNA were purified as described by Marmur (1961) and Malamy et al. (1972), respectively. DNA concentrations were determined spectrophotometrically, using the relationship 1:0 OD260 = 0:05 mg DNA ml⁻¹.

Endonuclease restriction of DNA. DNA was prepared for restriction by the method outlined in Maniatis et al. (1982). Restriction endonucleases with their appropriate buffers were used as recommended by the supplier.
Analysis of fepA mutants of E. coli

Table 1. E. coli strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB1515</td>
<td>azi-6 fhuA23 lacY1 leu-6 mtl-1 proCl4 purE42 rpsL109 thi-1 trpE38 tss-67</td>
<td>E. coli Genetic Stock Center</td>
</tr>
<tr>
<td>AN90</td>
<td>entD purE* transductant of AB1515</td>
<td>I. G. Young</td>
</tr>
<tr>
<td>AN93</td>
<td>entE purE* transductant of AB1515</td>
<td>I. G. Young</td>
</tr>
<tr>
<td>AN117</td>
<td>entF purE* transductant of AB1515</td>
<td>I. G. Young</td>
</tr>
<tr>
<td>RV</td>
<td>NlaC74</td>
<td>Malamy et al. (1972)</td>
</tr>
<tr>
<td>UT400, UT500, UT700, UT1500, UT2300, UT4400, UT5600, UT6100</td>
<td>fepA derivatives of RW193</td>
<td>McIntosh et al. (1979)</td>
</tr>
<tr>
<td>Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pACYC184</td>
<td>Cm'Tc' pACYC184 with an 11 kb insert carrying fepB fepC</td>
<td>Chang &amp; Cohen (1978)</td>
</tr>
<tr>
<td>pCP111</td>
<td>pACYC184 with a 7 kb insert carrying entABCEG</td>
<td>Pierce &amp; Earhart (1986)</td>
</tr>
<tr>
<td>pCP410</td>
<td>pBR322 with a 4.5 kb insert carrying dnaY</td>
<td>Pickett et al. (1984)</td>
</tr>
<tr>
<td>pDM1</td>
<td>pBR322 with a 2.3 kb insert containing ompT</td>
<td>Mullin et al. (1984)</td>
</tr>
<tr>
<td>pGGC110</td>
<td>fepC* Bal31 deletion derived from pCP111</td>
<td>Rupprecht et al. (1983)</td>
</tr>
<tr>
<td>pJP1543</td>
<td>entE<em>B+G</em> derivative of pCP410 constructed by Bal31 activity</td>
<td>Pierce &amp; Earhart (1986)</td>
</tr>
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<td>pLH18</td>
<td>entA<em>C</em> derivative of pCP410 obtained by Bal31 digestion</td>
<td>This laboratory</td>
</tr>
<tr>
<td>pLH1521</td>
<td>entA<em>C</em> derivative of pCP410 obtained by Bal31 digestion</td>
<td>This laboratory</td>
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<td>pML22</td>
<td>pBR322 with a 0.8 kb insert containing entY</td>
<td>Lundrigan &amp; Earhart (1984)</td>
</tr>
<tr>
<td>pMS101</td>
<td>pBR322 with an 11 kb insert carrying entD fepA fes entF</td>
<td>Laird &amp; Young (1980)</td>
</tr>
<tr>
<td>pPC11</td>
<td>fes* subclone of pMS101</td>
<td>Coderre &amp; Earhart (1984)</td>
</tr>
<tr>
<td>pRS31</td>
<td>pSC101 with an inserting traSTD1 IS3, Tc'</td>
<td>Skurray et al. (1976)</td>
</tr>
</tbody>
</table>

Enzyme reactions were terminated by heating at 65 °C for 5 min and were monitored for completion by agarose gel electrophoresis.

Electrophoresis of DNA. Agarose gel electrophoresis was performed using horizontal slab gels prepared by the method of McDonell et al. (1977). A 1-0 μg sample of restricted DNA was added to each well and DNA fragments were subsequently visualized by addition of 0-1 ml ethidium bromide (5-0 mg ml⁻¹) to the buffer. Gels were photographed with a Polaroid MP-4 camera, using type 55 Polaroid Land film. In order to prepare highly specific hybridization probes, it was often necessary to electroelute DNA fragments into wells filled with E buffer (Maniatis et al., 1982). Agarose gel was removed from the eluted sample by centrifugation prior to DNA precipitation.

Blot hybridizations. DNA to be hybridized was first electrophoresed in agarose gels. Fragments were transferred onto either nitrocellulose (Southern, 1975) or Zeta probe blotting membranes (Bio-Rad Bulletin 1110). Hybridization probes were labelled with [32P]dCTP by the procedure outlined in the Bio-Rad Nick Translation protocol. Labelled DNA was separated from unincorporated nucleotides by chromatography through a Sephadex G-50 column as described by Maniatis et al. (1982). DNA bound to membranes was hybridized with 32P-labelled probes overnight at 68 °C (Maniatis et al., 1982). Washes were also at 68 °C, as follows: 30 min in 500 ml 2 × SSC/0.5% SDS; 30 min in 500 ml 2 × SSC/0.1% SDS; 2 h in 500 ml 0-1 × SSC/0.5% SDS; and 1 h in 500 ml 0-1 × SSC/0.5% SDS (1 × SSC is 0-15 M-NaCl, 0-015 M-trisodium citrate, pH 7-0). Occasionally Zeta probe membranes were rehybridized as outlined in the Bio-Rad Bulletin 1110.

Assays. Enterobactin esterase activity was measured qualitatively as previously described (Pierce et al., 1983). Enterobactin synthase was assayed by following the conversion of [14C]serine into DBS-containing compounds (Bryce & Brot, 1972) in extracts prepared according to Luke & Gibson (1971). Enzymic complementation assays were performed by mixing extracts from each of the deletion strains with those from strains harbouring point mutations in enterobactin synthase genes and noting synthase activity. Protein concentrations were determined by the Lowry procedure, with bovine serum albumin as a standard, and enterobactin was estimated using the phenolate assay of Arnow (1937).
Table 2. Enterobactin synthase activity

The results are expressed as nmol L-[14C]serine converted to DBS-containing compounds in 15 min (mg protein)-1. They are means of at least two determinations.

<table>
<thead>
<tr>
<th>Source of supplementary extract</th>
<th>AN90 (entD)</th>
<th>AN93 (entE)</th>
<th>AN117 (entF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsupplemented extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB1515</td>
<td>9.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>RW193</td>
<td>10.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AN90</td>
<td>2.1</td>
<td>7.0</td>
<td>8.2</td>
</tr>
<tr>
<td>AN93</td>
<td>2.5</td>
<td>7.0</td>
<td>9.0</td>
</tr>
<tr>
<td>AN117</td>
<td>2.4</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>UT400</td>
<td>2.3</td>
<td>7.1</td>
<td>1.8</td>
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<tr>
<td>UT500</td>
<td>6.7</td>
<td>6.5</td>
<td>5.2</td>
</tr>
<tr>
<td>UT700</td>
<td>12.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>UT1500</td>
<td>2.1</td>
<td>5.4</td>
<td>2.1</td>
</tr>
<tr>
<td>UT2300</td>
<td>2.0</td>
<td>5.6</td>
<td>3.0</td>
</tr>
<tr>
<td>UT6100</td>
<td>2.8</td>
<td>5.5</td>
<td>2.3</td>
</tr>
</tbody>
</table>

ND, Not determined.

RESULTS

Enzymic analyses of fepA deletions

The extent of the fepA deletions with respect to other genes of the enterobactin cluster was examined by enzyme assay. Enterobactin synthase, a multienzyme complex composed of the entD, entE, entF, and entG gene products, converts serine and dihydroxybenzoic acid to enterobactin; the presence of functional entD, entE and entF genes in strains bearing deletions was tested (Table 2). The enterobactin synthase activity of strains AN90, AN93 and AN117, which have point mutations in relevant ent genes, was approximately 25% or less than that of control strains (AB1515 and RW193), and extracts from these mutants enzymically complemented one another. The results show that four deletion strains (UT400, UT1500, UT2300, and UT6100) lack functional entD and entF genes, whereas the remaining two strains (UT500 and UT700) are entD+ and entF+. These results are consistent with previous data: (i) UT500 produces a truncated FepA protein, and (ii) UT700 is able to synthesize enterobactin when provided with dihydroxybenzoic acid (McIntosh et al., 1979). Also, although no FepA polypeptide can be detected in UT700 (McIntosh, 1978), the fepA lesion has normal cotransduction frequencies with purE and entA (McIntosh et al., 1979). The results also demonstrate that the right-hand (clockwise) endpoint of the larger mutations lies between entF and entE, as all strains tested were entE+.

Ferrienterobactin esterase activity in the deletion mutants was consistent with the synthase assay data in that only UT500 and UT700 were fes+ (data not shown). The four genes at the left end of the enterobactin cluster (entD, fepA, fes and entF) have apparently been lost in the large deletion strains.

Involvement of IS3 in deletion formation

Several approaches were employed to test the idea that an IS3 was responsible for the prevalence of fepA deletions. First, IS3 probes were prepared from λlac5S7-MS505 (λlac) (Malamy et al., 1972) and pRS31 (Skurray et al., 1976). Each probe was then tested for its ability to hybridize with the chromosomal DNA present in three plasmids that amongst them contained all ent cluster genes. Surprisingly, with both probes homology was observed between ent cluster DNA and non-IS3 DNA of the probes. Thus, λlac hybridized to each insert (Fig. 2) and pRS31 bound to the bacterial DNA of plasmids pCP111 and pMS101 (data not shown). These results indicated that sequences other than IS3 present on the probes should be tested for binding. Probes prepared from pUC9, which like λlac contains the lacPOZ region, and pSC101, which is the vector used in the construction of pRS31, provided evidence that binding
of lacPOZ and pSC101 DNA to ent cluster DNA was responsible for the homologies observed with the \( \lambda \)plac and pRS31 probes, respectively.

A highly specific IS3 probe was made by isolating and nick-translating the 0.8 kb internal fragment produced by HindIII digestion of IS3. No significant hybridization occurred when this probe was incubated with ent cluster DNA.

The second approach used to determine if IS3 played a role in fepA deletion formation involved analysing whole chromosomal digests for IS3. If IS3 were involved, the size of fragments containing IS3 would be altered in the deletion mutants. Accordingly, chromosomal DNA from AB1515 and three mutants was digested with EcoRI or EcoRV and subsequently hybridized with the 0.8 \( \text{kb} \) IS3 probe. The binding patterns verified the lack of IS3 involvement in formation of these deletions (Fig. 3). Except for a minor alteration in UT400 (Fig. 3b, lane C), the IS3-binding patterns of the mutants were identical to those of AB1515.

**Physical mapping of deletions**

The entEBGCA region was intact in the deletion strains. Nick-translation of plasmids pLH18 (entEBG) and pLH1521 (entCA) produced probes that hybridized to an 11.6 \( \text{kb} \) HindIII fragment present in all strains (data not shown). This fragment corresponds to that present in a plasmid (pMS112) constructed by Laird et al. (1980) and shown to carry entEBGCA. These data are consistent with the enzymic complementation results.

Additional experiments resolved the right-hand endpoints of the deletions more precisely. A probe prepared from pJP1543 (fepC) hybridized with HindIII digests of chromosomal DNA in all cases; UT6100 and UT4400 had the wild-type 6.6 \( \text{kb} \) fragment but UT2300 and UT5600 had fragments of 12 and 10.8 \( \text{kb} \), respectively (data not shown), indicating that the deletions in these latter two strains extended beyond the HindIII site located between entF and fepC. Similar experiments were done with EcoRI and EcoRV chromosomal digests and a probe prepared from the 6.6 \( \text{kb} \) HindIII fragment (fepBfepC) of pCP111 (Fig. 4). The EcoRI site between fes and entF had been deleted in strains UT400 and UT2300 as the fepBfepC region was carried on 21 and
Fig. 3. Autoradiogram of $^{32}$P-labelled IS3 DNA hybridized to a Southern blot of EcoRI- or EcoRV-digested chromosomal DNA. DNA from AB1515 (A), UT2300 (B), UT400 (C) and UT6100 (D) was digested with either EcoRI (a) or EcoRV (b) and hybridized to the 0.8 kb IS3 HindIII fragment of pRS31. Fragment sizes (kb) are indicated on the left.

Fig. 4. Autoradiogram of hybridization of $^{32}$P-labelled fepBfepC DNA to chromosomal DNA. EcoRI (a) and EcoRV (b) digests of chromosomal DNA from AB1515 (A), UT2300 (B), UT400 (C) and UT6100 (D) were hybridized to the 6.6 kb HindIII fragment of pCP111 that carries the fepBfepC genes. The sizes (kb) of the fragments detected are indicated on the left.
**Analysis of fepA mutants of E. coli**

Fig. 5. Hybridization of envi to chromosomal DNA. Lanes A, D and G are EcoRI digests, B, E and H are BamHI digests, and C, F and I are EcoRI/BamHI double digests. Plasmid pML22 was nick-translated and hybridized to the following bacterial DNAs: (a) RW193 (A–C); UT5600 (D–F); UT400 (G–I). (b) UT2300 (A–C); UT4400 (D–F); UT6100 (G–I). Lanes J and K show hybridization to control plasmids pML22 and pGGC110, respectively.

20.5 kb fragments in UT400 and UT2300, respectively, rather than the approximately 11 kb fragment present in wild-type E. coli K12 (Fig. 1; Laird et al., 1980; Pierce & Earhart, 1986). [The presence of an approximately 18 kb fragment in AB1515 indicates that this strain lacks the EcoRI site between fepB and entA; the corresponding region in the deletion strains is from AN92 (entA) by transduction (Leong & Neilands, 1976).] UT6100 may have the typical 11 kb fragment, but lack of resolution in this region of the gel makes a definitive statement impossible. The fepBfepC probe contained sequences complementary to four EcoRV fragments (4.0, 1.8, 1.0 and 0.24 kb) (Fig. 1); the three largest fragments were detectable by hybridization (Fig. 4b, lane A). By noting which fragments were lost or altered (Fig. 4), approximate endpoints for the deletions in UT400, UT2300, and UT6100 were determined.

Hybridizations of Southern blots of both EcoRI and EcoRV digests of UT400, UT2300 and UT6100 with the 2.8 kb EcoRI fragment of pPC11 demonstrated that fes DNA was absent in these strains (data not shown). These data agreed with the enzyme analyses (see above).

Additional mapping was done to determine the left-hand endpoints of the deletions. The dispensable genes ompT and envi map at approximately 12.9 min (Lundrigan & Earhart, 1984); probes to each were prepared from pGGC110 and pML22, respectively. With the exception of UT2300, all the mutant strains tested lacked envi (Fig. 5) and ompT (data not shown). The dnaY gene, which is essential and which maps at approximately 12.5 min (Henson et al., 1979; Bachmann, 1983), was, as anticipated, present in all strains (data not shown). The probe for dnaY was prepared from pDM1.

**DISCUSSION**

The chromosomal region containing the enterobactin gene cluster is unusual in that it is enriched for IS elements (Timmons et al., 1983), contains a defective lambdoid prophage.
sequences responsible and their locations in the bacterial DNA. The same studies will determine if an additional gene exists in the clockwise termini region.

pMSlO1, it is possible the IS3 probes pCPl11 and pMSlOl. Because there is overlap between the bacterial DNA in pCPl11 and pMSlOl, it is possible the IS3 probes pCP410, pCPl11 and pMSlOl (Fig. 2), and (ii) pSClOl DNA and the insert DNA of pSClOl, which apparently have small deletions (McIntosh et al., 1979; Table 2), the deletion strains have clockwise endpoints in the fepC region. Recently, Ozenberger et al. (1987) presented preliminary evidence for a gene (fepD) between fepC and fepB; the relatively small variation among these endpoints may indicate that this is a crucial gene. Concerning the left-hand endpoints, hybridization results were consistent with previous gel analyses for the OmpT protein (Earhart et al., 1979). However, results with enY, whose product is involved in thermoregulation of porins, showed two discrepancies with earlier data (Lundrigan & Earhart, 1984). UT400 and UT6100 were shown previously to have normal porin thermoregulation despite the present evidence that they lack enY. We have no explanation for this inconsistency; it may be a reflection of the complex nature of porin regulation, which involves a multiplicity of environmental factors and regulatory genes. Hybridization results with UT2300, UT400 and UT5600 are in agreement with the previous thermoregulation studies.

The cause of the fepA deletion mutations remains undetermined. Prokaryotic translocatable elements can stimulate the frequency of spontaneous deletions (e.g. Reif & Saedler, 1975; Kleckner et al., 1979) and we previously suggested (McIntosh et al., 1979) that the IS3 in the vicinity of the enterobactin region was responsible for the prevalence of fepA deletions. However, in the present study we found no evidence to support this idea: (i) probes specific for IS3 failed to detect such a sequence in the enterobactin gene cluster, and (ii) when the IS3 patterns of three fepA deletion mutants were analysed, two proved to be identical to that of the wild-type. In a third mutant, UT400, IS3-containing EcoR1 fragments appeared normal but an EcoRV fragment containing IS3 was altered (Fig. 3). Most probably, the counterclockwise end of the UT400 fepA deletion terminates near an IS3 that is flanked on its right by a close EcoR1 site and on its left by an EcoRV site. Results with the IS3 probe also indicate that AB1515, like other E. coli K12 strains (Deonier et al., 1979), contains five copies of this IS.

Deletions caused by translocatable elements generally have one variable and one constant endpoint, the latter at the site of the transposon. Among the fepA deletion mutants screened, a variety of right and left endpoints were observed (Fig. 1b). The hybridization mapping of the large deletions showed that at least three right-hand endpoints were present in the several mutants tested. Data regarding the left-hand endpoints was of necessity less definitive because of the paucity of well-mapped and cloned genes in the 12-13 min region of the E. coli chromosome. However, there are at least two endpoints, and transductional data (Earhart et al., 1979), in conjunction with the data on clockwise endpoints, suggest that a variety of left-hand endpoints exist. This argues that neither IS3 nor any normal translocatable element in the vicinity, such as the IS5 insert in mmpC (Highton et al., 1985; Blasband et al., 1986), was involved in their formation. Although not definitive, the endpoint results also suggest that the enterobactin gene cluster of E. coli K12 is not bordered by IS elements. Bernardi & Bernardi (1984) demonstrated that altered IS elements can lead to deletion series with no fixed endpoint, albeit with a reduced frequency. If a transposon is responsible for the fepA deletions it probably contains a mutated sequence.

Unexpected homologies were discovered (i) between lacPOZ DNA and bacterial DNA present in pCP410, pCP111 and pMS101 (Fig. 2), and (ii) pSC101 DNA and the insert DNA of pCP111 and pMS101. Because there is overlap between the bacterial DNA in pCP111 and pMS101, it is possible the IS3 probes Aplac and pRS31 exhibit homology to only two and one regions of enterobactin DNA, respectively. The significance, if any, of these homologies is unknown but DNA sequencing studies now under way should permit us to identify the sequences responsible and their locations in the bacterial DNA. The same studies will determine if an additional gene exists in the clockwise termini region.
We thank Dr S. Payne for assistance with the hybridization experiments and Drs A. J. Clark, J. R. Walker and M. H. Malamy for providing strains used in this study.

This work was supported by Public Health Service grants AI-17794 and AI-22203 from the National Institute of Allergy and Infectious Diseases.

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