Homologous regions of the *Salmonella enteritidis* virulence plasmid and the chromosome of *Salmonella typhi* encode thiol:disulphide oxidoreductases belonging to the DsbA thioredoxin family

José M. Rodríguez-Peña, Isabel Alvarez, Magdalena Ibáñez and Rafael Rotger

Author for correspondence: Rafael Rotger. Tel: +34 1 394 17 43. Fax: +34 1 394 17 45.
e-mail: rotmifar@eucmax.sim.ucm.es

Departamento de Microbiología II, Facultad de Farmacia, Universidad Complutense, E28040 Madrid, Spain

The nucleotide sequence relatedness between the chromosome of *Salmonella typhi* and the virulence plasmid of *Salmonella enteritidis* was investigated using short DNA probes of $< 2$ kb covering the whole virulence plasmid sequence. Only one homologous region was detected. This region was subsequently cloned and partially sequenced. Sequences closely related to the *pefi* gene and the ORFs *orf7, orf8* and *orf9*, which are located downstream of the fimbrial *pef* operon of the *Salmonella typhimurium* virulence plasmid, were detected. Sequencing of the cloned *S. typhi* DNA fragment also revealed identity with genes of the fimbrial *sef* operon characterized in the chromosome of *S. enteritidis*. These nucleotide sequences mapped upstream of the *S. typhi* chromosomal region homologous to the *S. enteritidis* virulence plasmid. The general organization of the cloned *S. typhi* chromosomal fragment was similar to the fimbriae-encoding region of the *S. typhimurium* virulence plasmid. The deduced product of *orf8* in the *S. typhimurium* virulence plasmid, as well as those of the corresponding ORFs in the homologous region of the *S. typhi* chromosome and in the *S. enteritidis* virulence plasmid (designated *dlt* and *dlp*, respectively), appeared to be related to the thioredoxin family of thiol:disulphide oxidoreductases. The *dlp* gene was able to complement the DTT-sensitive phenotype, the inability to metabolize glucose 1-phosphate and the low alkaline phosphatase activity of a *dsbA* mutant of *Escherichia coli*. The *dlt* gene partially complemented the lack of alkaline phosphatase activity, but not the other mutant phenotypes. The products of both genes could be detected using the T7 RNA polymerase promoter expression system. The estimated molecular masses of the products of the *dlt* and *dlp* genes by SDS-PAGE were 26 and 23 kDa, respectively, the first being in agreement with the deduced amino acid sequence and the latter, somewhat smaller. The processing of a possible leader peptide in the Dlp protein, but not in the Dlt protein, could be responsible for this difference. The Dlp protein appeared as a doublet band on SDS-PAGE, which is characteristic of the oxidized and reduced states of this kind of protein.

Keywords: *Salmonella typhi, Salmonella enteritidis, virulence plasmid, dsbA, dlt and dlp*

The EMBL accession numbers for the sequences of *dlt* and *dlp* reported in this paper are X94325 and X94326, respectively.
INTRODUCTION

Several serovars of Salmonella, including those most frequently isolated from salmonellosis in humans, harbour virulence-related plasmids, whose role in pathogenesis remains as yet unclear (Gulig et al., 1993). Gulig & Doyle (1993) demonstrated that the virulence plasmid of Salmonella typhimurium enhances its growth rate when infecting the mouse. A possible role for the virulence plasmid in systemic infection in humans has been inferred from the higher frequency of plasmid-bearing isolates from blood in comparison to the strains obtained from faeces (Montenegro et al., 1991; Fierer et al., 1992). However, Salmonella typhi, the serovar causing typhoid fever in man, lacks a virulence plasmid.

As the virulence plasmid has also been found integrated in the chromosome of S. typhimurium, Korpela et al. (1989) examined this possibility in S. typhi by DNA hybridization. They demonstrated hybridization between a 15 kb fragment of the S. typhimurium plasmid and chromosomal DNA of S. typhi. However, they did not characterize the homologous regions in either the S. typhi chromosome or the virulence plasmid of S. typhimurium.

The present study was undertaken to characterize the hybridizing region of the S. typhi chromosome. By means of Southern blot hybridization using smaller plasmid-derived DNA probes of < 2 kb, we analysed the nucleotide sequence similarity between the virulence plasmid of Salmonella enteritidis strain 82139 (Buisán et al., 1994) and chromosomal DNA of S. typhi. However, they did not characterize the homologous regions in either the S. typhi chromosome or the virulence plasmid of S. typhimurium.

Recombinant DNA techniques and nucleotide sequence analysis. Unless otherwise stated, recombinant DNA techniques were performed as described by Sambrook et al. (1989). Plasmid DNA was extracted from Salmonella strains by the method of Kado & Liu (1981) and from E. coli by the procedure of Birnboim & Doly (1979). When necessary for sequencing purposes, plasmid DNA was purified with the Qiagen system. Total DNA from Salmonella was obtained as described by Ausubel et al. (1993). Southern and colony blot hybridizations were carried out using digoxigenin labelling and chemiluminescent detection systems (Boehringer Mannheim); hybridization reactions were done at 68 °C. DNA sequencing was carried out in an automated laser fluorescent sequencer (ALF, Pharmacia) using the phage M13 universal- and reverse-sequencing fluorescein-labelled primers (Pharmacia). In some cases, sequencing was completed using specific fluorescein-labelled primers synthesized in a Gene Assembler (Pharmacia) and designed to give overlapping reading in both strands. The nucleotide sequences obtained were compiled and analysed using the PC/GENE software (Intelligenetics). The search for similarities in nucleotide and amino acid sequences was done in the EMBL and GenBank databases using FASTA, BLITZ and BLAST software through the Internet.

Alkaline phosphatase assays. Alkaline phosphatase activities were measured essentially as described by Brickman & Beckwith (1975) and Gutierrez et al. (1987). Bacterial strains were grown overnight in P medium at 37 °C with shaking. Cells were collected from 1 ml culture by centrifugation and resuspended in 1 ml 10 mM Tris/HCl (pH 8.0), 0.1 M NaCl; 0.5 ml of this suspension was added to 0.5 ml 1 M Tris/HCl (pH 8.0). Then 50 µl 0.1 % (w/v) SDS and 50 µl chloroform were added. After agitation, 100 µl 0.4 % p-nitrophenol phosphate (Sigma) in 1 M Tris/HCl (pH 8.0) was added, and the reaction mixture was incubated at 28 °C until a yellow colour developed. The reaction was then stopped by the addition of 100 µl 1 M KH₂PO₄ and the samples were read in a Beckman DU640 spectrophotometer. Units of alkaline phosphatase were calculated as indicated by Brickman & Beckwith (1975).

DTT sensitivity assays. Complementation of the dsbA mutation in E. coli strain SS140 was detected by sensitivity to 10 mM DTT, which was the minimum concentration that inhibited the growth of E. coli strain SS140 (DsbA-) without affecting growth of E. coli wild-type strain CU141 (DsbA+). The assay was done in wells of microtitre plates containing about 8 x 10⁶ cells and 10 mM DTT in 100 µL medium. The plate lids were sealed with Teflon sealing tape to prevent DTT evaporation and bacterial growth was observed after 14 h incubation at 37 °C.

Expression of the cloned genes. The T7 phage promoter of plasmid pBluescript SK(+) was used to express the cloned dlt genes in E. coli strain BL21(DE3) (Table 1). This strain contains a copy of the T7 RNA polymerase gene inserted under the control of the lacZ promoter and, therefore, inducible by IPTG. In the presence of rifampicin, the bacterial RNA polymerase is inhibited, and only the genes inserted in plasmid pBluescript downstream of the T7 phage promoter can be expressed (Studier & Moffatt, 1986). The 1.5 kb NarI-SmaI fragments of plasmids pST93 and pMJ003 (Fig. 2) were subcloned in pBluescript SK(+) to give rise to the plasmids pST94 (carrying the dlt gene) and pSE3 (carrying the dlp gene). The expression studies were carried out essentially as described by Sambrook et al. (1989), except that 30 min after the induction of the promoter of the T7 RNA polymerase gene with 1 mM IPTG, rifampicin was added to a final

METHODS

Bacterial strains, plasmids and media. The bacterial strains used in this work are shown in Table 1. Escherichia coli strain DH5α was used as the recipient in cloning experiments, with pBluescript SK (+) (Stratagene) as a cloning vector. The plasmid YEP357R was used to obtain translational fusions with a lacZ gene lacking promoter sequences (Myers et al., 1986). All strains were routinely grown at 37 °C in Luria broth (LB) with shaking or on Luria agar plates supplemented, when necessary, with ampicillin (100 µg ml⁻¹), IPTG (50 µg ml⁻¹) and X-Gal (50 µg ml⁻¹). DM minimal salt medium (Neidhardt et al., 1974) supplemented with 10 mM glucose 1-phosphate was used to test the ability to grow using this substrate as sole source of carbon. P medium, containing peptone (Difco; 10 g L⁻¹) and NaCl (5 g L⁻¹) (pH 7.4), was used in the alkaline phosphatase assays (Ito et al., 1983).
plasmid pFM82139, which had been previously cloned with the aim of detecting the possible relatedness of any fragments obtained from the S. enteritidis virulence plasmid probes.

**RESULTS**

Detection and cloning of a region from the chromosome of S. typhi hybridizing with the S. enteritidis virulence plasmid

With the aim of detecting the possible relatedness of any region of the chromosome of S. typhi with the nucleotide sequence of the virulence plasmid, we used small fragments (< 2 kb) derived from the virulence plasmid of S. enteritidis as DNA probes. All the HindIII fragments obtained from the S. enteritidis virulence plasmid pFM82139, which had been previously cloned in plasmid vector pBluescript (Buisán et al., 1994), were further digested with several restriction endonuclease mixtures (EcoRI/PstI, EcoRI/XhoI, HaeIII/HindIII) or HaeIII. These fragments (< 2 kb) were labelled with digoxigenin and hybridized against chromosomal DNA from S. typhi strain 5866 digested with restriction endonuclease BamHI.

The only DNA fragments of the S. enteritidis virulence plasmid which hybridized with chromosomal DNA of S. typhi strain 5866 were those contained in recombinant plasmid pMJ003 (Table 1). These DNA probes hybridized with a 13 kb BamHI fragment of S. typhi chromosomal DNA (Fig. 1). With the purpose of cloning this fragment, we eluted from a 0.7% agarose gel the chromosomal fragments of 10–15 kb generated by restriction endonuclease BamHI and ligated them to the cloning vector pBluescript SK(+) . Transformants of E. coli DH5α containing recombinant plasmids were obtained by co-transformation. The only DNA fragments of the S. enteritidis virulence plasmid which hybridized with chromosomal DNA of S. typhi strain 5866 were those contained in recombinant plasmid pMJ003 (Table 1). These DNA probes hybridized with a 13 kb BamHI fragment of S. typhi chromosomal DNA (Fig. 1). With the purpose of cloning this fragment, we eluted from a 0.7% agarose gel the chromosomal fragments of 10–15 kb generated by restriction endonuclease BamHI and ligated them to the cloning vector pBluescript SK(+) . Transformants of E. coli DH5α containing recombinant plasmids were obtained by co-transformation.

<table>
<thead>
<tr>
<th><strong>Table 1. Bacterial strains and plasmids used in this work</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strain/plasmid</strong></td>
</tr>
<tr>
<td><strong>Strains</strong></td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em> 82139</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em> 366</td>
</tr>
<tr>
<td><em>Salmonella dublin</em> 19</td>
</tr>
<tr>
<td><em>Salmonella typhi</em> 5866</td>
</tr>
<tr>
<td><em>Salmonella montevideo</em> 6173</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> C53</td>
</tr>
<tr>
<td><em>Escherichia coli</em> DH5α</td>
</tr>
<tr>
<td><em>Escherichia coli</em> BL21(DE3)</td>
</tr>
<tr>
<td><em>Escherichia coli</em> SS140</td>
</tr>
<tr>
<td><em>Escherichia coli</em> CU141</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
</tr>
<tr>
<td>pBluescript SK(+)</td>
</tr>
<tr>
<td>pFM82139</td>
</tr>
<tr>
<td>pMJ003</td>
</tr>
<tr>
<td>pMJ003</td>
</tr>
<tr>
<td>pSE3</td>
</tr>
<tr>
<td>pST93</td>
</tr>
<tr>
<td>pST94</td>
</tr>
</tbody>
</table>

* The Spanish National Centre for Notifiable Diseases.

concentration of 150 μg ml⁻¹, except in the controls. Following 150 min incubation, 1 ml samples of the culture were labelled with 2 μCi (74 kBq) [³⁵S]methionine for 10 min. The cells were collected by centrifugation and suspended in electrophoresis sample buffer. The solubilized proteins were examined by SDS-PAGE using separating and stacking gels containing 15% (w/v) and 5% (w/v) acrylamide, respectively.
was the *S. enteritidis* virulence plasmid DNA insert of recombinant plasmid pMJ003 (Fig. 2).

The nucleotide sequence of the cloned *S. typhi* chromosomal region partially corresponds to the fimbrial *sef* genes

Searches in the nucleotide sequence data libraries revealed a high degree of identity (96–97%) among the partial sequences of plasmid pST93 and the *sefA*, *sefC* and *sefD* genes of the *sef* operon previously described in the *S. enteritidis* chromosome, which are required for the production of SEF14 and SEF18 fimbriae (Clouthier *et al.*, 1993, 1994; Collinson *et al.*, 1996).

The region of nucleotide sequence similarity (70–98%) between recombinant plasmid pST93 and plasmid pMJ003 containing the 5.8 kb *HindIII–SmaI* fragment of the *S. enteritidis* virulence plasmid pFM82139 begins after the *XhoI* site and extends 116 bp beyond the second *EcoRV* site, with a total size of 2.6 kb (Fig. 2). This region of plasmid pMJ003 matches a part of the *pef* locus in the virulence plasmid of *S. typhimurium* that encodes synthesis of fimbriae, namely the ORFs *pefl*, *orf7*, *orf8* and *orf9*, described by Friedrich *et al.* (1993). Nucleotide sequence similarity with the *pefC* gene (96%) was also found adjacent to the left *HindIII* site in plasmid pMJ003 (Fig. 2), suggesting that the same organization of this fimbrial operon occurs in both *S. enteritidis* and *S. typhimurium* virulence plasmids (Friedrich *et al.*, 1993). Partial sequencing of plasmid pST93 also showed a substantial sequence similarity to the ORFs *pefl*, *orf7*, *orf8* and *orf9*. As a whole, the genetic organization of the cloned *S. typhi* chromosomal fragment, including the *sef*-related region, was strikingly similar to that of the *pef* region of the *S. typhimurium* plasmid (Friedrich *et al.*, 1993).

The rest (6.2 kb) of the nucleotide sequence of plasmid pST93 did not show significant sequence relatedness to any registered sequence, with the exception of two small regions (Fig. 2). One of them, of 131 bp, is 89% identical to an upstream sequence of the *sinIM* gene of *Salmonella infantis*, which encodes a DNA cytosine methyltransferase (Karreman & Waard, 1988). The second region, of 200 bp, showed 77% identity with the *mcrD* gene, adjacent to the *mcrBC* restriction system genes in *E. coli* but not described in *Salmonella* (Burland *et al.*, 1995).

The *S. enteritidis* virulence plasmid and the homologous locus from the *S. typhi* chromosome could encode a protein related to the DsbA family of disulphide oxidoreductases

No significant relationship between the nucleotide sequence of *orf8* from the virulence plasmid of *S. typhimurium* and the sequences existing in the DNA sequence data libraries has been reported (Friedrich *et al.*, 1993). However, we observed some amino acid sequence similarity between the putative gene product...
complement several defective phenotypes of 

In order to clarify whether these ORFs could actually

In the cloned homologous fragments of the S.

been reported to be essential in this type of enzyme (for

orfs (Shigella flexneri) [here named DsbA(S);

 TcpG protein from 

Comparison of the DNA nucleotide

Fig. 3. Comparison of the DNA nucleotide sequences of the DsbA-like encoding ORFs from the plasmids of S. typhimurium (orf8) (Friedrich et al., 1993) and S. enteritidis (dlp), and from the chromosome of S. typhi (dlt). Only the changes in the nucleotide sequences of the dlp and dlt genes are shown; gaps introduced by the program to optimize the alignment are represented by dashes. The possible -35 and -10 regions, ribosome-binding site (RBS) and the start and stop codons are underlined, according to the published data (Friedrich et al., 1993).

(a)

(b)

of the translated orf8 ORF and the members of the DsbA family of thiolsulphide oxidoreductases, mainly around the preserved motif Cys-X-X-Cys, which has been reported to be essential in this type of enzyme (for a review see Bardwell, 1994). The deduced amino acid sequences of the putative products of the corresponding ORFs in the cloned homologous fragments of the S. typhi chromosome (plasmid pST93) and the virulence plasmid of S. enteritidis (plasmid pMJ003) exhibited the same similarity to the DsbA family of enzymes. We named these ORFs dlt and dlp, respectively. The alignments of the nucleotide and amino acid sequences corresponding to these genes and those of the other bacteria are shown in Figs 3 and 4.

In order to clarify whether these ORFs could actually encode disulphide oxidoreductase enzymes, we tried to complement several defective phenotypes of E. coli strain SS140 (DsbA⁻) (Kishigami et al., 1995). Plasmids pMJ003 or pSE3 or both, each of which contains the dlp gene (Fig. 2), were able to restore the wild-type phenotypes of E. coli strain CU141 (Table 2). On the other hand, plasmid pST93 or its subclone pST94 or both, each of which contains the dlt gene (Fig. 2), partially complemented the lack of alkaline phosphatase activity but failed to restore the resistance to 10 mM DTT and the ability to grow on glucose 1-phosphate as sole source of carbon (Table 2).

As the failure of the dlt gene to efficiently complement the phenotype of the DsbA⁻ strain of E. coli could have been due to a lack of expression, we constructed gene fusions of both the dlp and dlt genes with a promoter-deficient lacZ gene (Fig. 2). Similar β-galactosidase activities on X-Gal plates were detected in both cases. The products of the dlp and dlt genes were detected after their overexpression using the T7 phage promoter (Fig. 5). The product of the dlt gene is a protein of 26 kDa, a
Table 2. Complementation of the *E. coli* *dsbA* mutation with plasmids carrying the chromosomal *S. typhi* *dlt* gene or the *S. enteritidis* virulence plasmid *dlp* gene

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Growth with 10 mM DTT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Growth on glucose 1-phosphate medium&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Alkaline phosphatase&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CU141 (<em>dsbA&lt;sup&gt;+&lt;/sup&gt;</em>)</td>
<td>+</td>
<td>+</td>
<td>100.0%</td>
</tr>
<tr>
<td>SS140 (<em>dsbA</em> mutant)</td>
<td>−</td>
<td>−</td>
<td>40.5%</td>
</tr>
<tr>
<td>SS140/pBluescript SK(+)</td>
<td>−</td>
<td>−</td>
<td>42.6%</td>
</tr>
<tr>
<td>SS140/pMJ003 (<em>dlp&lt;sup&gt;+&lt;/sup&gt;</em>)</td>
<td>+</td>
<td>+</td>
<td>122.0%</td>
</tr>
<tr>
<td>SS140/pSE3 (<em>dlp&lt;sup&gt;−&lt;/sup&gt;</em>)</td>
<td>+</td>
<td>ND</td>
<td>102.6%</td>
</tr>
<tr>
<td>SS140/pST93 (<em>dlt&lt;sup&gt;+&lt;/sup&gt;</em>)</td>
<td>−</td>
<td>−</td>
<td>78.8%</td>
</tr>
<tr>
<td>SS140/pST94 (<em>dlt&lt;sup&gt;−&lt;/sup&gt;</em>)</td>
<td>−</td>
<td>ND</td>
<td>83.8%</td>
</tr>
</tbody>
</table>

ND, Not determined.

<sup>a</sup>Resistance to DTT was assayed in LB medium with 10 mM DTT.

<sup>b</sup>Ability to grow with glucose 1-phosphate as the sole source of carbon was assayed in DM minimal medium with 10 mM glucose 1-phosphate.

<sup>c</sup>Alkaline phosphatase activities were determined by the method of Brickman & Beckwith (1975) and expressed as a percentage of the activity detected in *E. coli* strain CU141, to which was assigned a value of 100%.

---

Fig. 5. Expression of the *dlp* and *dlt* genes under the influence of the T7 phage promoter. An autoradiogram of the dried gel is shown; numbers on the left indicate the positions and molecular masses in kDa of the pre-stained protein molecular size standards (Amersham). Lanes 1 and 2 correspond to cell extracts of *E. coli* strain BL21(DE3) containing the plasmid pSE3 (*dlp*) and lanes 3 and 4 correspond to cell extracts of *E. coli* carrying the plasmid pST94 (*dlt*; see Fig. 2). The bacteria corresponding to lanes 1 and 3 were not treated with rifampicin.

Fig. 6. Southern blot analysis of total DNA isolated from the following strains and digested either with restriction endonucleases *BamHI* (lanes 1, 3, 5, 6 and 7) or *HindIII* (lanes 2 and 4): *S. enteritidis* strain 366 (lanes 1 and 2), *S. enteritidis* strain 82139 (lanes 3 and 4), *S. dublin* strain 19 (lane 5), *S. typhimurium* strain C53 (lane 6) and *S. montevideo* strain 6173 (lane 7). The DNA was transferred to a nylon membrane and hybridized with the 409 bp *SmaI*–*Sacll* fragment of plasmid pST93 (see Fig. 2) labelled with digoxigenin as a probe. Sizes are indicated on the right in kb.

---

Fig. 5. Expression of the *dlp* and *dlt* genes under the influence of the T7 phage promoter. An autoradiogram of the dried gel is shown; numbers on the left indicate the positions and molecular masses in kDa of the pre-stained protein molecular size standards (Amersham). Lanes 1 and 2 correspond to cell extracts of *E. coli* strain BL21(DE3) containing the plasmid pSE3 (*dlp*) and lanes 3 and 4 correspond to cell extracts of *E. coli* carrying the plasmid pST94 (*dlt*; see Fig. 2). The bacteria corresponding to lanes 1 and 3 were not treated with rifampicin.

molecular mass that agrees with that deduced from the amino acid sequence of the gene product (24700 Da). The expression of the *dlp* gene consistently gave two adjacent protein bands on SDS-PAGE. This observation agrees with the reported data about the DsbA protein of *E. coli*, which also gave two bands in SDS-PAGE corresponding to the oxidized and reduced states of the cysteine residues (Kishigami *et al.*, 1995). The estimated
molecular mass in SDS-PAGE of the Dlp protein was about 23 kDa, whereas that deduced from the amino acid sequence of the gene product was 24400 Da.

**Presence of dlp-related sequences in the chromosome of other serotypes of Salmonella**

Some Salmonella serovars were examined by Southern blot hybridization using the 409 bp Smal–SacII fragment of plasmid pST93 as a DNA probe (Fig. 2). We detected hybridization with chromosomal DNA fragments of 9 or 5 kb (corresponding to the digestion with endonucleases BamHI or HindIII, respectively) from S. enteritidis strain 366, which lacks the virulence plasmid, and Salmonella dublin strain 19, which harbours a virulence plasmid (Fig. 6). Hybridizing fragments of the same size were detected in total DNA from S. enteritidis strain 82139, together with others (of 12.6 and 10.3 kb, corresponding to the digestion with BamHI or HindIII, respectively) which should belong to the virulence plasmid. It is noteworthy that neither the plasmid-cured strain C53 of S. typhimurium, nor Salmonella montevideo strain 6173, yielded hybridization reactions (Fig. 6).

**DISCUSSION**

The lack of a virulence plasmid in S. typhi is striking, considering its essential role in systemic infections caused by other serovars. However, most of the plasmid-borne genes, except the spv operon, are of marginal or unknown importance in pathogenesis. The finding of virulence-plasmid-related genes in the chromosome of S. typhi could be indicative of the importance of their roles. However, we did not find homologous regions additional to that reported by Korpela et al. (1989), despite the use of shorter plasmid-derived DNA probes which should have allowed us to detect single genes.

Analysis of the DNA fragments cloned from the chromosome of S. typhi and from the plasmid of S. enteritidis showed that the homologous regions were adjacent to fimbriae-encoding regions in these strains. The present report presents the first direct evidence of the presence and organization of the sefA, sefC and sefD genes in S. typhi. The structural subunit of the SEF18 fimbriae, which is encoded by the sefD gene, was previously detected immunologically in S. typhi, but there is no evidence of the functionality of these fimbriae as adhesins (Clothier et al., 1994). In the case of the SEF14 fimbriae, the sefA gene, which encodes their structural subunit, was previously detected by DNA hybridization in S. typhi, but its expression could not be confirmed by agglutination tests (Turcotte & Woodward, 1993). Therefore, the role of these genes in the adhesion of S. typhi remains unproven.

The similarity of its deduced amino acid sequence, and its complementation of a dsbA mutant of E. coli, allows us to propose the inclusion of the product of the dlp gene in the thioredoxin family of thiol-disulphide oxido-reductases (Bardwell, 1994; Loferer & Hennecke, 1994). The variable degree of complementation of the dsbA mutation with the dlt gene could be explained by a different specificity or activity of the enzyme, or by a different cellular localization. It is known that the DsbB protein is required in E. coli for the reoxidation of DsbA (Bardwell, 1994), but the oxido-reduction system could be different in S. typhi. The lack of visualization of the putative oxidized and reduced forms of the Dlt protein (Fig. 5) could support the latter hypothesis, but it would be necessary to know the actual oxido-reduction state of the protein. The estimated molecular mass of the Dlp protein of 23 kDa is as would be expected after processing of a signal peptide, as occurs with other DsbA-like proteins. However, the deduced amino acid sequences of the Dlp and Dlt proteins clearly differ from those of the DsbA proteins of E. coli, Sh. flexneri and V. cholerae in the region corresponding to the leader peptide (Fig. 4). Moreover, we could not detect a consensus sequence for a signal peptide processing site either in the Dlt protein or in the Dlp protein. The deduced amino acid sequence of the amino-terminal region of the Dlp and Dlt proteins appears to be much more similar to that of the orf8 gene product than to those of the other DsbA proteins (Fig. 4). It has been reported than the deduced amino acid sequence of the orf8 gene product of the virulence plasmid of S. typhimurium reveals a signal sequence of 21–23 amino acids typical of a lipoprotein (Friedrich et al., 1993). It is tempting to explain the differences in molecular masses and in activities of the Dlp and Dlt proteins by the lack of signal peptide processing in the latter protein; in fact, the deduced amino acid sequence of the Dlp protein is much more closely related to that deduced from orf8 around the predicted cleavage site than to the Dlt amino acid sequence (Fig. 4).

The proximity of the dlp and dlt genes to the fimbrial operons pef and sef could suggest a function of these genes in the processing of some component of these fimbriae. Moreover, we did not detect a reaction with a dlt gene probe in the two strains of Salmonella belonging to serogroups other than serogroup D (namely S. typhimurium and S. montevideo), in agreement with the report of the presence of sefA only in members of serogroup D (Turcotte & Woodward, 1993; Collinson et al., 1996). To our knowledge, there is no information about the presence of the pef operon in the virulence plasmid of S. dublin, and we did not detect apparent hybridization of the dlt gene probe with DNA of this plasmid (Fig. 6). The hybridization of the dlt gene probe with DNA of the S. enteritidis chromosome indicates that this serovar could have a second gene encoding thiol-disulphide oxido-reductase associated with the sef operon. The synthesis of Pef fimbriae is independent of ORFs 7, 8, 9 and 11, but the adhesiveness of these fimbriae could not be proved (Friedrich et al., 1993); in the same way, the correct assembly of SEF14 and SEF18 fimbriae appears to be dependent on some unknown
accessory genes (Clouthier et al., 1993, 1994). It should also be borne in mind that the expression of these fimbriae has always been performed in a DsbA+ background, which could disguise the need for expression of the dlt or dlp genes or of orf8. It is noteworthy that the nucleotide sequence identity between plasmid pST93 and the pef operon includes pefl, the putative regulator (Friedrich et al., 1993), which suggests that an undescribed regulatory gene of the chromosomal sef operon could also exist. These possibilities are currently under investigation.

ACKNOWLEDGEMENTS

This work was supported by grant AL194-0509 from Comisión Interministerial de Ciencia y Tecnologia. J. M. Rodríguez-Peña and I. Alvarez were aided by fellowships from Ministerio de Educación y Ciencia. The authors would like to acknowledge M. Isabel García-Saiz for her sequencing work, Dr K. Ito and Dr S. Kishigami, Kyoto University, for their advice and for supplying the SS140 and CU141 strains, and Dr F. Norel, Institut Pasteur, for supplying the C53 strain.

REFERENCES


Received 31 July 1996; revised 10 October 1996; accepted 8 November 1996.