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Identification and Sequence Analysis of *lpfABCDE*, a Putative Fimbrial Operon of *Salmonella typhimurium*

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A chromosomal region present in *Salmonella typhimurium* but absent from related species was identified by hybridization. A DNA probe originating from 78 min on the *S. typhimurium* chromosome hybridized with DNA from *Salmonella enteritidis*, *Salmonella heidelberg*, and *Salmonella dublin* but not with DNA from *Salmonella typhi*, *Salmonella arizonae*, *Escherichia coli*, and *Shigella* serotypes. Cloning and sequence analysis revealed that the corresponding region of the *S. typhimurium* chromosome encodes a fimbrial operon. Long fimbriae inserted at the poles of the bacterium were observed by electron microscopy when this fimbrial operon was introduced into a nonpiliated *E. coli* strain. The genes encoding these fimbriae were therefore termed *lpfABCDE*, for long polar fimbriae. Genetically, the *lpf* operon was found to be most closely related to the *fim* operon of *S. typhimurium*, both in gene order and in conservation of the deduced amino acid sequences.

All members of the family *Enterobacteriaceae* express fimbriae, or pili, which allow them to attach to host surfaces. This attachment is the first step in the colonization of their preferred host niche. The various fimbrial adhesins were originally distinguished by their receptor specificity. Hemagglutination of erythrocytes was used as a criterion to define three fimbrial classes (for a recent review, see reference 14). Fimbriae whose agglutination is prevented by coincubation with D-mannose were designated type 1 fimbriae (41). Type 2 fimbriae, a nonhemagglutinating fimbrial class, were later found to resemble nonadhesive variants of type 1 fimbriae (20, 36, 43). Finally, pili able to agglutinate animal erythrocytes only after the erythrocytes have been treated with tannic acid were designated type 3 fimbriae (22, 23). This early classification was supported by data on the morphological characteristics of these fimbriae. Type 1 and type 2 fimbriae have a width of 7 to 8 nm and display a channeled rodlike appearance due to the arrangement of subunits around a hollow core (43). Type 3 fimbriae are 4 to 5 nm in diameter and lack an axial hole on the basis of electron microscopic examination (42). Recently, thin fimbriae (3 to 4 nm) with a unique curly morphology in members of the *Enterobacteriaceae* have been described (4, 44). A comparison of sequence data obtained from several fimbrial systems with similar morphologies revealed that the major fimbrial subunits of curly fimbriae show a high degree of sequence conservation. These fimbriae are therefore now considered to form a distinct fimbrial class. The designation GVVPQ has been proposed, in reference to a short amino acid sequence conserved at the N terminus of their major fimbrial subunit (19). Type 1, type 2, type 3, and GVVPQ fimbriae have the common characteristic that their fimbrial subunits contain signal sequences which are cleaved by signal peptidase I. In contrast, a fifth enterobacterial class of pili, termed type 4 fimbriae, has been shown to use a different export system for translocation of its fimbrial subunits across the cytoplasmic membrane. Fimbrial subunits of type 4 fimbriae contain signal sequences which are recognized and cleaved by type IV prepilin signal peptidase (for a recent review, see reference 54). As with that for the other fimbrial types, this classification has been supported by several morphological characteristics which distinguish enterobacterial type 4 fimbriae from other fimbrial classes. While type 1, type 2, type 3, and GVVPQ fimbriae from enterobacteria have a length of 0.5 to 2 μm and are arranged peritrichously around the bacterial cell, enterobacterial type 4 fimbriae are polar and have a length of 10 to 20 μm (28).

Here we describe a fimbrial operon of *Salmonella typhimurium* termed *lpf*. Introduction of the *lpf* operon into *Escherichia coli* results in the expression of fimbriae with a morphology similar to that of type 4 fimbriae. However, sequence analysis indicated that the *lpf* operon is not closely related to that expressing type 4 fimbriae but rather shows similarity to that expressing *S. typhimurium* type 1 fimbriae.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. *E. coli* ORN172 has been described previously (59). *E. coli* DH5α and LE392 were purchased from Gibco BRL and New England Biolabs, respectively. *S. typhimurium* 14028, 6994, 7823, and LT2 were obtained from the American Type Culture Collection (ATCC). *E. coli* W3110, *Salmonella dublin* LANE, and *S. typhimurium* SL1344 and SR-11 were from laboratory stocks. Clinical isolates of *Shigella sonnei*, *Shigella boydii*, *Shigella dysenteriae*, enteroinvasive *E. coli*, enterotoxigenic *E. coli*, enteropathogenic *E. coli*, *Salmonella arizonae*, *Salmonella typhi*, *Salmonella heidelberg*, and *Salmonella enteritidis* were obtained from the State of California Health Laboratory or the County of San Diego Health Laboratory and have been described previously (34). *Shigella flexneri* BS176 and M90T have been described elsewhere (61).

All bacteria were cultured aerobically in Luria-Bertani broth (5 g of yeast extract per liter, 8 g of tryptone per liter, and 5 g of NaCl per liter) or on plates (Luria-Bertani broth containing 15 g of agar per liter) at 37°C. Antibiotics, when required, were included in the culture medium or plates at the following concentrations: carbenicillin, 100 mg/liter; kanamycin, 60 mg/liter; tetracycline, 15 mg/liter; and streptomycin, 50 mg/liter. Analytical grade chemicals were purchased from Sigma. All enzymes were purchased from Boehringer Mannheim.

Pulsed-field gel electrophoresis. The preparation of agarose-embedded chromosomal DNA for pulsed-field gel electrophoresis was based on a protocol from Liu and Sanderson (35). In brief, cells were harvested from 5 ml of an early-log-phase bacterial culture, resuspended in 0.5 ml of prewarmed (37°C) cell suspension buffer (10 mM Tris-HCl [pH 7.2], 20 mM NaCl, 100 mM EDTA), and mixed with 0.5 ml of 2% InCert agarose (InCert) which was dissolved in phosphate-buffered saline and precooled to 37°C. The mixture was poured into molds (Bio-Rad) and allowed to solidify. The molds were digested with lysozyme (1 mg/ml; New England Biolabs) in a volume of 7.5 ml for 1 h at room temperature in buffers provided by the manufacturer. The lysozyme solution was replaced by 7.5 ml of proteinase K (1 mg/ml; New England Biolabs) in buffer provided by the

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manufacturer and incubated at 55°C for 48 h with shaking. Agarose-embedded DNA prepared in this manner can be stored in 7.5 ml of TE buffer (10 mM Tris-HCl [pH 7.2], 5 mM EDTA) at 4°C for several months without degradation.

For restriction digests, an agarose block equivalent to about 0.05 to 0.1 ml was incubated for 15 min at room temperature in 0.2 ml of 1× KGB buffer (38) in which sodium acetate was replaced by potassium acetate. The buffer was replaced with 1× KGB buffer containing 20 U of *Xba*I, and the samples were incubated for 4 h at 37°C. The agarose blocks were then loaded on an agarose gel for pulsed-field gel electrophoresis.

Pulsed-field gel electrophoresis of a 1% agarose gel was performed in 0.5% Tris-borate-EDTA at 200 V with pulse lengths of 6 to 150 s for 6 h, 6 to 12 s for 6 h, 26 to 36 s for 6 h, and 120 to 180 s for 6 h with a CHEF-DRII apparatus from Bio-Rad.

Recombinant DNA techniques. Plasmid DNA was isolated with ion exchange columns from Qiagen. Standard methods were used for restriction endonuclease analyses, ligation and transformation of plasmid DNA, and isolation of chromosomal DNA from bacteria (39, 48). The construction of the gene bank from *S. typhimurium* ATCC 14028 in the cosmid vector pLAFRII has been described elsewhere (34). Subclones of cosmid inserts were constructed with the vector pBluescript SK+ (52). Sequencing was performed by the dideoxy chain termination method (49) with an AutoRead Sequencing Kit (Pharmacia) and an ALF automatic sequenator or according to a protocol of Kraft et al. (33) with α-^{35}S-dATP (Amersham) being used for labeling.

Southern hybridization. Southern transfer of DNA onto a nylon membrane was performed as previously described (48). The labeling of DNA probes, hybridization, and immunological detection were performed with the DNA labeling and detection kit (nonradioactive) from Boehringer Mannheim. The DNA was labeled by random primed incorporation of digoxigenin-labeled dUTP. Hybridization was performed at 65°C in solutions without formamide. For Southern hybridization with chromosomal DNA of different bacterial species, two 15-min washes were performed under nonstringent conditions at room temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS). For Southern hybridization with cosmids of a gene bank or with DNA separated on a pulsed-field gel, a nonstringent wash (10 min at room temperature in 2× SSC–0.1% SDS) and a stringent wash (30 min at 65°C in 0.2× SSC–0.1% SDS) were performed. Hybrids were detected by an enzyme-linked immunoassay with an antibody conjugate (anti-digoxigenin alkaline phosphatase conjugate) and the substrate AMPPD [3-(2′-spiroademantane)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxethane; Boehringer Mannheim]. The light emitted by the dephosphorylated AMPPD was detected by X-ray film.

Preparation of fimbrial crude extracts. The protocol used for the preparation of fimbrial crude extracts was based on a protocol by van Zijderveld et al. (58). In brief, bacteria were grown in 100 ml of Luria-Bertani broth to mid-log phase and were harvested by centrifugation (3,000 × *g* for 10 min). After the bacteria were resuspended in 10 ml of 75 mM NaCl–0.5 mM Tris-HCl (pH 7.0), they were incubated at 60°C for 30 min and then pelleted by centrifugation (3,000 × *g* for 10 min). The supernatant was transferred to a fresh tube, and solid ammonium sulfate was added to reach a final saturation of 60%. The mixture was incubated for 1 h at 4°C with stirring. Proteins were harvested by centrifugation (40,000 × *g* for 30 min), and the supernatant was discarded. The fimbrial crude extracts were resuspended in 0.1 ml of 75 mM NaCl–0.5 mM Tris-HCl (pH 7.0) and dialyzed overnight at 4°C against 0.1 M Tris-HCl, pH 7.0. Fimbrial crude extracts were boiled for 10 min in sample buffer containing 4% SDS and separated on a 15% Tricine-SDS-polyacrylamide gel (51). After electrophoresis, the proteins were visualized by silver staining.

Electron microscopy. Bacteria were grown overnight in a static culture and were allowed to adhere to a Formvar-coated grid for 2 min. The bacteria were fixed with 0.1% glutaraldehyde in sodium cacodylate buffer (100 mM, pH 7.4) for 1 min. The grid was rinsed with water, and the fimbriae were negatively stained with 0.5% (wt/vol) aqueous uranyl acetate, pH 4.6, for 30 s. The grids were allowed to dry before they were analyzed by electron microscopy.

Computer analysis. The nucleotide sequences were compared with nonredundant updates of SWISS-PROT, PIR(R), and GenPept with the program blastX and were compared with nonredundant updates of GenBank and EMBL with the program blastN (3). The nucleotide sequences were further analyzed with the PC/GENE software package.

Nucleotide sequence accession number. The nucleotide sequence of the 7,065-bp DNA fragment reported on in this paper has been assigned the GenBank accession number U18559. The nucleotide sequence of a region of the *E. coli* K-12 chromosome which corresponds to 78 min on the *S. typhimurium* genome has been determined previously. It has been assigned GenBank accession number U00039.

RESULTS

Identification and cloning of a *Salmonella*-specific chromosomal region from *S. typhimurium*. Several short chromosomal DNA fragments from *S. typhimurium* were obtained by inverse PCR from chromosomal DNA of strains carrying transposon Tn*10* insertions at different map locations (8). In order to identify regions on the *S. typhimurium* chromosome which were not present in related members of the *Enterobacteriaceae*, we used these DNA fragments as probes in a Southern hybridization with DNA from several related species. Under the conditions used, a hybridization signal should be obtained if a DNA fragment showed a more than 65% nucleotide sequence identity with a probe. A 210-bp DNA probe (the insert of plasmid pMS10) flanking the Tn*10* insertion of strain MS1521 hybridized with DNA from *Salmonella choleraesuis* subsp. *choleraesuis*, including the serotypes *S. enteritidis*, *S. heidelberg*, *S. dublin*, and several frequently used strains of serotype *S. typhimurium* (for current nomenclature on the *Salmonella* genus, see reference 31). No hybridization signal was detected with DNA from the *S. choleraesuis* subsp. *choleraesuis* serotype *S. typhi*, *S. choleraesuis* subsp. *arizonae*, several *E. coli* isolates, and *Shigella* serovars (Fig. 1). A 317-bp DNA fragment containing an internal part of the *S. typhimurium purD* gene (8) was used to probe a second blot containing chromosomal DNA from the same enterobacterial species. The *stn* gene, which is located only 1.4 kb downstream of *purD* (13, 45), has been shown to be duplicated in *S. typhimurium* (12). The two bands hybridizing with the *purD* probe in DNA from *Salmonella* serotypes indicate that this duplication includes part of the *purD* gene. Unlike the pMS10 probe, the *purD* probe gave a hybridization signal with all strains tested (Fig. 1). These data indicated that the insert of plasmid pMS10 originates from an *S. typhimurium* region which is present in serotypes of *S. choleraesuis* subsp. *choleraesuis* except serovar *S. typhi*.

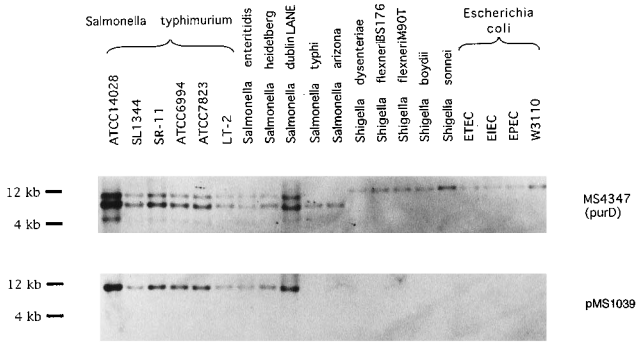

FIG. 1. Southern blot of chromosomal DNA from enterobacteria digested with *Eco*RI, separated on an agarose gel, and hybridized with part of the *S. typhimurium purD* gene (MS4347) and with part of the *lpf* operon (pMS1039). The designations of the bacterial strains used for the preparation of the chromosomal DNA are indicated above the blots. The positions of the DNA fragments used as size standards are given on the left. ETEC, enterotoxigenic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli.*

The corresponding region of the *S. typhimurium* chromosome was cloned from a previously described cosmid bank constructed in pLAFRII and propagated in *E. coli* LE392 (34). The cosmid bank was spread on plates, and 300 colonies were picked and grown up individually overnight. Cosmid DNA was prepared from 30 pools, each containing 10 overnight cultures, and digested with *Eco*RI, separated on an agarose gel, and hybridized with probe pMS10. The DNA of two pools hybridized with probe pMS10. The 20 cosmids included in these two pools were then isolated individually and digested with *Eco*RI and *Pst*I, and the hybridization was repeated with probe pMS10. Each pool contained one cosmid, termed pMS1000 and pMS1001, respectively, which gave a hybridization signal corresponding to a 2-kb *Eco*RI-*Pst*I DNA fragment (data not shown). The 2-kb *Eco*RI-*Pst*I fragment was cloned into the vector pBluescript SK+ (52) to give rise to plasmid pMS1039

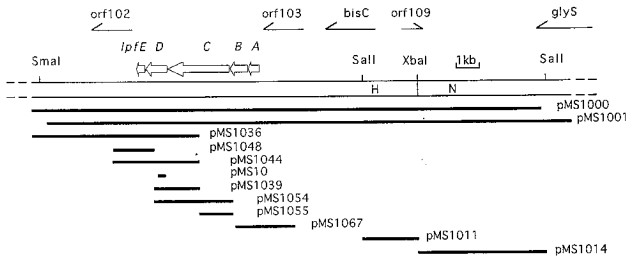

FIG. 2. Restriction map of an *S. typhimurium* chromosomal region located around 78 min on the genetic map. The region shown contains the ends of *Xba*I fragment H (H) and *Xba*I fragment N (N) from *S. typhimurium* (34). The inserts of cosmids pMS1000 and pMS1001 are shown below. The positions of the inserts contained in several subclones (pMS1036, pMS1048, pMS1044, pMS10, pMS1039, pMS1054, pMS1055, pMS1067, pMS1011, and pMS1014) are given at the bottom of the figure. The positions and orientations of the genes *lpfABCDE* are indicated by outlined arrows above the restriction map. The approximate positions of genes showing short stretches of homology to those of *E. coli* are indicated at the top of the figure by thin arrows and the designation of the *E. coli* homolog.

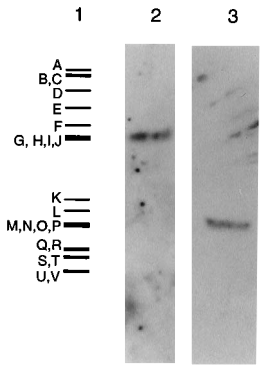

FIG. 3. Southern blot of chromosomal DNA from *S. typhimurium* 14028 digested with *Xba*I, separated by pulsed-field agarose gel electrophoresis, and hybridized with probe pMS1039 (lane 2) or probe pMS1014 (lane 3). The positions and designations (according to reference 34) of the *Xba*I fragments of *S. typhimurium* 14028 are given in lane 1.

(Fig. 2). In order to confirm the hybridization data obtained with probe pMS10, the 2-kb insert of pMS1039 was used as a probe in a Southern hybridization with DNA from several enterobacterial species (Fig. 1). The hybridization pattern obtained with probe pMS1039 was found to be identical to the one obtained with probe pMS10.

Restriction map and physical map location of the cloned *Salmonella*-specific region. In order to construct a restriction map, cosmids pMS1000 and 1001 were digested with the restriction enzymes *Xba*I, *Sal*I, *Sma*I and with combinations of these. After separation on an agarose gel, DNA fragments were hybridized with probe pMS10 (data not shown). In addition, a 5.7-kb *Sal*I-*Xba*I fragment from pMS1000 was cloned into pBluescript SK+ (pMS1014) and used as a probe for hybridization with the same blot (data not shown). The information obtained from these experiments allowed the construction of a restriction map of cosmids pMS1000 and 1001 (Fig. 2).

Cosmids pMS1000 and pMS1001 contained an *Xba*I site located a distance of about 10 kb from the insert of pMS10 (Fig. 2). The restriction endonuclease *Xba*I cuts the *S. typhimurium* chromosome only 23 times, creating fragments of between 18 and 800 kb which have been designated alphabetically according to their size. The map positions of all *Xba*I sites on the *S. typhimurium* chromosome have been determined so that a macrorestriction map of the *S. typhimurium* genome can be constructed (35). The Tn*10* insertion in strain MS1521 has previously been mapped to 78 min on the *S. typhimurium* chromosome, close to an *Xba*I site located between *Xba*I fragments N and H (8). In order to determine whether the *Salmonella*-specific region defined by pMS10 and pMS1039 is located on *Xba*I fragment N or H, chromosomal DNA of *S. typhimurium* was digested with *Xba*I, separated by pulsed-field electrophoresis, and hybridized with probe pMS1039 and probe pMS1014. A hybridization signal corresponding to *Xba*I fragment G, H, I, or J was obtained with probe pMS1039, while probe pMS1014 gave a hybridization signal corresponding to *Xba*I fragment N, M, O, or P (Fig. 3). With the map location determined previously for the Tn*10* insertion in strain MS1521 (8), these data show that the inserts of pMS10 and pMS1039 originate from *Xba*I fragment H at 78 min on the *S. typhimurium* genome (Fig. 2).

Sequence analysis of the *Salmonella*-specific DNA region. The nucleotide sequence of a 7,065-bp DNA region was determined (Fig. 4). The region was found to encode five open reading frames, designated *lpfABCDE*, which are transcribed counterclockwise from the *S. typhimurium* chromosome (Fig. 2). In addition, the sequence contained the beginning and the end of two further open reading frames, designated *orf1* and *orf2*, respectively (Fig. 4).

The positions of open reading frames *lpfABCD* and *-E* on the nucleotide sequence and the sizes of the predicted polypeptide are given in Table 1 and Fig. 4. All predicted polypeptides were found to contain typical signal sequences (Table 1).

Sequence comparison with *E. coli* defines the endpoints of the *Salmonella*-specific DNA region. The nucleotide sequence of a region of the *E. coli* K-12 chromosome which corresponds to 78 min on the *S. typhimurium* genome was compared with the nucleotide sequence determined from pMS1000 in order to identify regions of DNA which are conserved between *E. coli* and *S. typhimurium* and those which are present in *S. typhimurium* only.

The deduced amino acid sequences of *orf1* and *orf2* shared 64 and 91% identities with the deduced amino acid sequences of the *E. coli* genes *orf103* and *orf102*, respectively. In *E. coli*, *orf103* and *orf102* are separated by 327 bp of noncoding DNA. In contrast, *orf1* and *orf2* are separated by a 6,053-bp DNA region which contains the open reading frames *lpfABCDE* (Fig. 5A). The GC content of open reading frames *lpfABCDE* is close to the GC content of 51 to 53% described for the entire *Salmonella* genome (Table 1) (24). However, this DNA region showed no homology to DNA from this region of the *E. coli* chromosome. The DNA-DNA homology of *E. coli* and *S. typhimurium* ends on one side close to the beginning of *orf2* (Fig. 5B) and on the other side close to the end of *orf1* (Fig. 5C). A short imperfect inverted repeat (13 bp with 11 matches) was found at the boundaries of the sequence homology in the *S. typhimurium* sequence (Fig. 5B and C). The above-mentioned results show that *orf1* and *orf2* of *S. typhimurium* are separated by a 6,053-bp region of DNA which is not present in *E. coli* and therefore confirm data obtained by hybridization with probes pMS10 and pMS1039 (Fig. 1).

In order to determine whether any other major differences in gene order between DNA present in pMS1000 or pMS1001 and the corresponding region of *E. coli* can be detected, the nucleotide sequences of short stretches of DNA were determined from the ends of plasmids pMS1011 and pMS1014 (Fig. 2). By comparison of the deduced amino acid sequences, *Salmonella* homologs of *bisC*, *orf109*, and *glyS* were found at

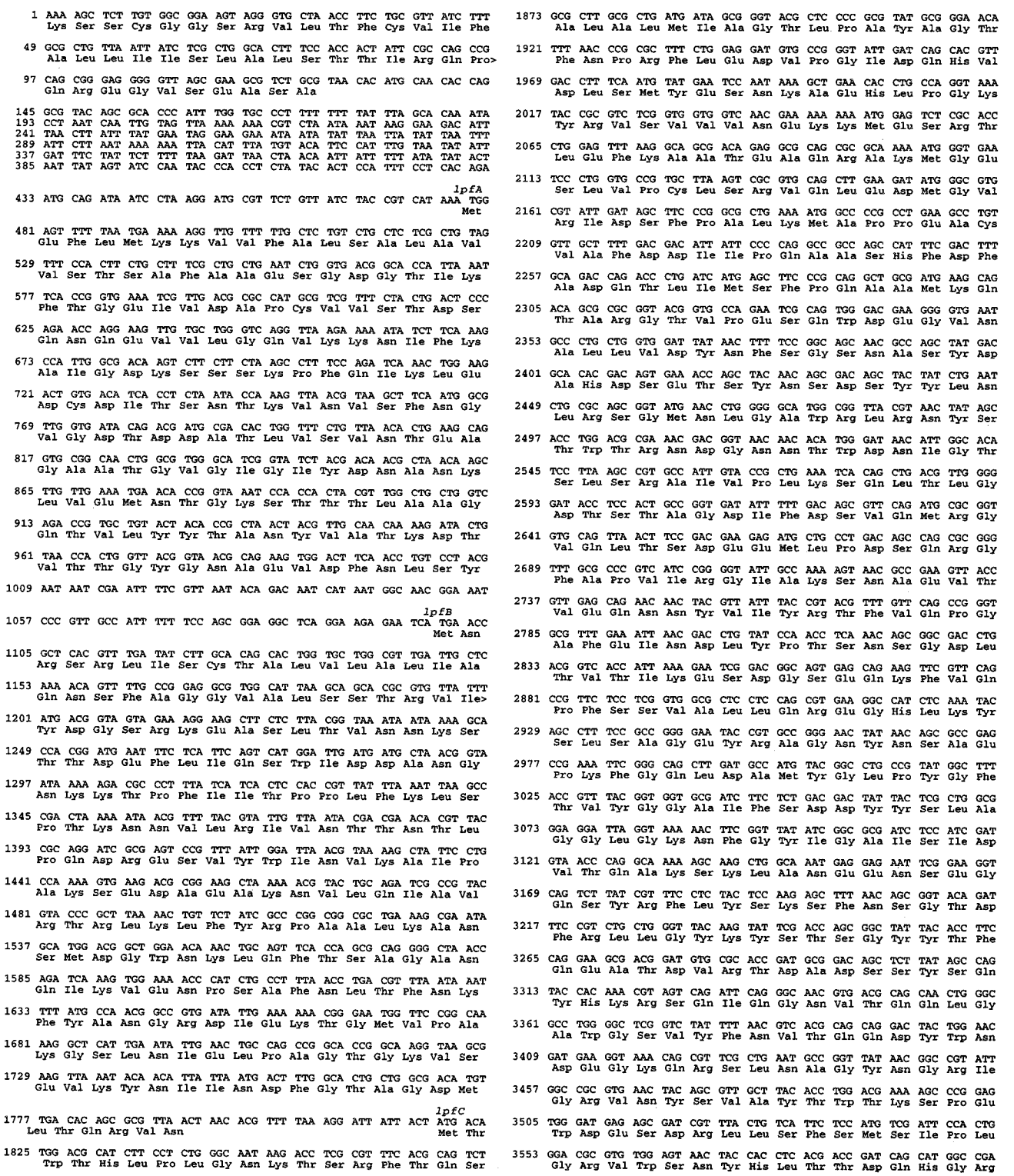

FIG. 4. Nucleotide sequence of a 7,065-bp DNA fragment encoding LP fimbriae. The positions and deduced amino acid sequences of *orf1*, *lpfA*, *lpfB*, *lpfC*, *lpfD*, *lpfE*, and *orf2* are indicated. The numbers on the left indicate the position of the first nucleotide in each line.

positions indicated in Fig. 2 (data not shown). These genes were found to be located at the same positions as the corresponding *E. coli* genes, indicating that no other large insertions are present in either species.

Homology of LpfABCDE to other fimbrial proteins. The deduced amino acid sequences of all five *lpf* genes showed significant homology to those of proteins from various bacterial fimbrial systems (Fig. 6). Furthermore, the gene order

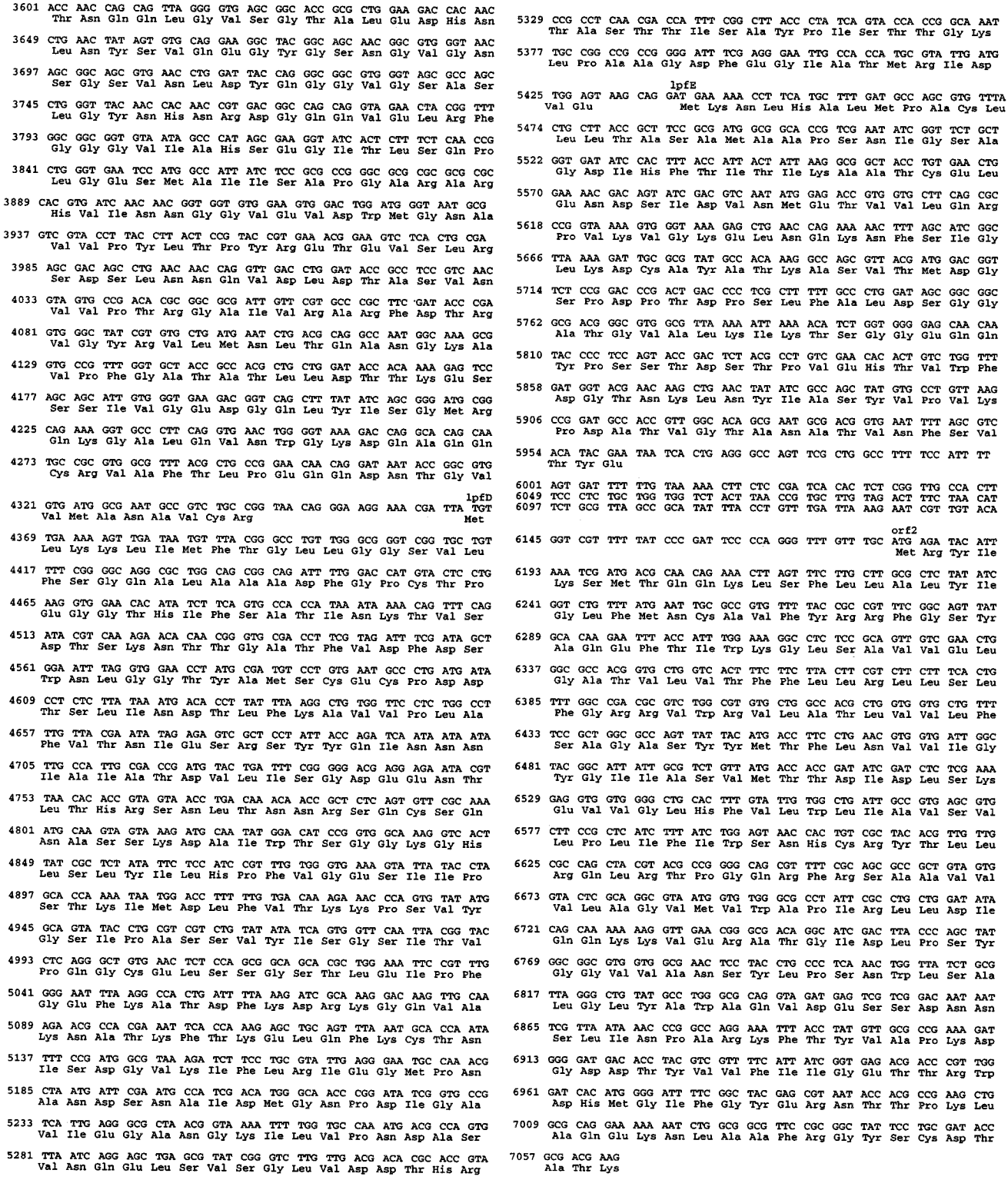

FIG. 4—*Continued.*

between *lpfABCDE* and the *fim* operon of *S. typhimurium* was found to be conserved (Fig. 6). These data strongly suggest that *lpfABCDE* constitutes a fimbrial operon. Start and stop codons of the genes *lpfBCDE* are separated by only a few base pairs; thus, this organization also substantiates the proposed operon structure (Fig. 4). A stem-loop structure marking a potential end of the operon was found downstream of *lpfE* (5′ stem bp 5974 to 5979; loop bp 5980 to 5983; 3′ stem bp 5984 to 5989;

TABLE 1. Sizes and positions of genes and gene products of the *lpf* operon

Open reading frame	Position (bp)[a]	GC content (%)	Length of polypeptide (amino acids)	Predicted signal sequence (amino acids)	Calculated mass of mature protein (Da)
lpfA	477–1010	47	179	1–24	16,138
lpfB	1098–1793	45	233	1–26	22,898
lpfC	1819–4344	55	843	1–21	90,158
lpfD	4365–5429	47	356	1–24	35,234
lpfE	5438–5962	51	175	1–20	16,335

[a] Position in the nucleotide sequence shown in Fig. 4.

$\Delta G(25°C) = -12.6$ kJ). For several fimbrial operons, a second stem-loop structure between the gene encoding the major fimbrial subunit and downstream genes has been found (7, 18, 26). Similarly, a stem-loop structure between *lpfA* and *lpfB* was found (5′ stem bp 1042 to 1052; loop bp 1053 to 1057; 3′ stem bp 1058 to 1068; $\Delta G(25°C) = -22.2$ kJ). By analogy, this suggests that *lpfA* may encode the major fimbrial subunit. This assumption is further supported by the sequence homology

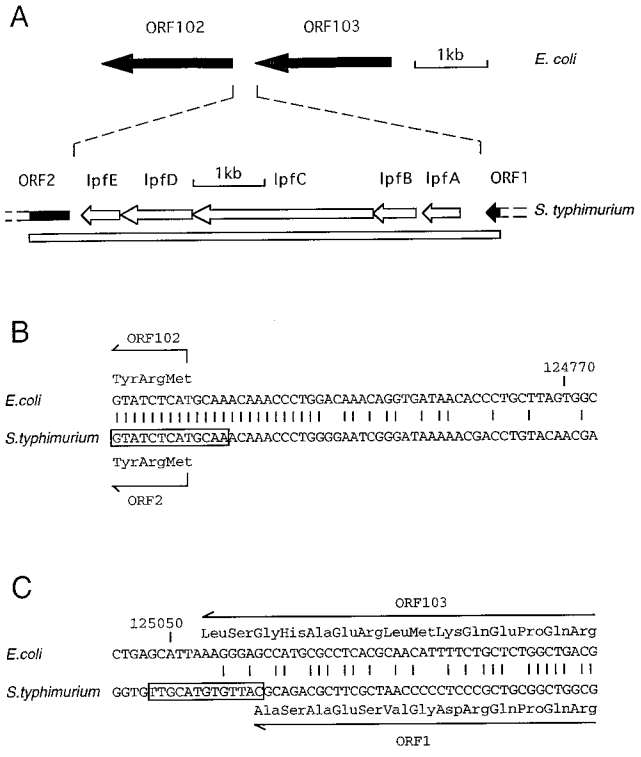

FIG. 5. (A) Comparison of the region of the *S. typhimurium* chromosome containing the *lpf* operon with the corresponding *E. coli* region. The positions of the genes are indicated by arrows. Solid arrows represent genes which are present in *E. coli* and *S. typhimurium*, while open arrows represent genes which are present only in *S. typhimurium*. The bar indicates the position of the nucleotide sequence shown in Fig. 4. The boundaries of sequence homology between DNA from *S. typhimurium* and *E. coli* are indicated by dashed lines. (B) Comparison of the nucleotide sequences at the left boundaries of sequence homology of *S. typhimurium* and *E. coli*. The start points of *orf2* and *orf102* are indicated by arrows. The numbers above the *E. coli* sequence refer to the position in the database entry U00039. The position of an inverted repeat found at the boundary of the sequence homology is boxed. (C) Comparison of the nucleotide sequences at the right boundaries of sequence homology of *S. typhimurium* and *E. coli*. The ends of *orf1* and *orf103* are indicated by arrows. The numbers above the *E. coli* sequence refer to the position in the database entry U00039. The position of an inverted repeat found at the boundary of the sequence homology is boxed.

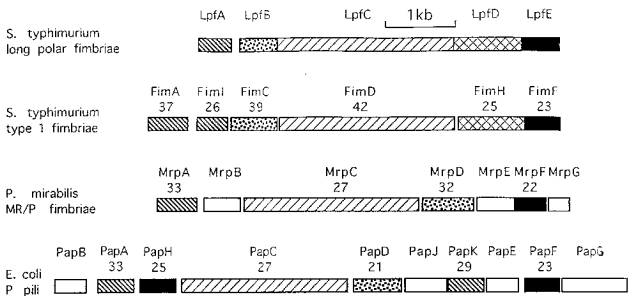

FIG. 6. Comparison of the deduced amino acid sequences and the gene orders of fimbrial operons from *E. coli* (*pap* operon), *Proteus mirabilis* (*mrp* operon), and *S. typhimurium* (*fim* operon) with those of the *lpf* operon. The position and length of a gene in an operon are indicated by bars. Open bars indicate genes which have no homologous partner in the *lpf* operon. Homologs to *lpf* genes in other fimbrial operons are indicated by identical bars. The numbers indicate the percent sequence identities between the deduced amino acid sequences of the *lpf* genes and homologs in other fimbrial operons.

found between LpfA and major subunits from other fimbrial systems (Fig. 7A and B). The sequence homology to other fimbrial systems suggests that LpfB, LpfC, and LpfE function as chaperonin, the usher, and the minor fimbrial subunit, respectively (Fig. 7C, D, and F). *S. typhimurium* FimH was the only homolog of LpfD found in the current protein database (Fig. 7E). So far, no function has been proposed for this protein.

Analysis of the *lpfA* promoter region. In order to identify potential regulatory sequences, the *lpfA* promoter region was assayed for the presence of GATC sites and direct or inverted repeats. Recently, phase variation of fimbrial operons mediated by differential methylation of GATC sites has been described (9, 57). However, no GATC sites were found in the promoter region of *lpfA*, making a regulation by this mechanism unlikely.

A 9-bp DNA sequence was found to form four direct repeats and one inverted repeat located between bp 263 and 388 in the *lpfA* promoter region (Fig. 8A). This sequence was also found to form an inverted repeat in the *fimA* promoter region of *S. typhimurium* and *S. typhi* (46, 47). The positions of these short repeats overlap a putative $\sigma 70$ promoter found upstream of *lpfA* (-35 sequence, bp 293 to 298; -10 sequence, bp 315 to 320). However, further experiments are necessary to establish a regulatory role for these repeats.

A inverted repeat was found to flank the open reading frame *lpfA* (Fig. 8B). The nucleotide sequence of this inverted repeat was similar to that of the inverted repeat which is thought to be involved in an inversion event controlling fimbrial phase variation in the *fim* operon of *S. typhimurium* and *S. typhi* (46, 47).

Expression of the *lpf* operon in *E. coli* and identification of long polar (LP) fimbriae. In order to determine whether pMS1000 encodes *S. typhimurium* fimbriae, this cosmid was introduced into nonfimbriated *E. coli* ORN172 (59). This strain carries a deletion in the *fim* operon and has been shown by electron microscopy not to express fimbriae. Fimbrial crude extracts from strains ORN172 and ORN172(pMS1000) were prepared, and the protein profiles were analyzed (Fig. 9). The fimbrial crude extract of strain ORN172(pMS1000) contained a band with an apparent molecular mass of 22 kDa which was not present in the crude extract of strain ORN172 (Fig. 9). The size of this protein was slightly larger than the calculated size for the major form of LpfA and LpfE (Table 1). The presence of proteins in a fimbrial crude extract is generally considered evidence for the expression of fimbriae. Therefore, these data

FIG. 7. Comparison of the primary structures of LpfA and FimA (A), LpfA and FimI (B), LpfB and FimC (C), LpfC and FimD (D), LpfD and FimH (E), and LpfE and FimF (F) by means of the program CLUSTAL. Identical amino acids are indicated by stars, and conservative amino acid changes are indicated by dots. Gaps introduced by the program to optimize the alignment are indicated by dashes.

supported the idea that *lpfABCDE* encode proteins involved in the biosynthesis of fimbriae, as suggested by the sequence homology of their gene products to those of the *fim* genes of *S. typhimurium.*

In order to detect expression of fimbriae directly, strains ORN172 and ORN172(pMS1000) were analyzed by electron microscopy. Fimbriae which were about 7 to 8 nm in width and which displayed a rodlike appearance were detected in strain ORN172(pMS1000) (Fig. 10) but not in strain ORN172 (data not shown). These morphological features are typical for enterobacterial type 1 and type 4 fimbriae. However, unlike type 1 fimbriae, the fimbriae detected on strain ORN172(pMS1000)

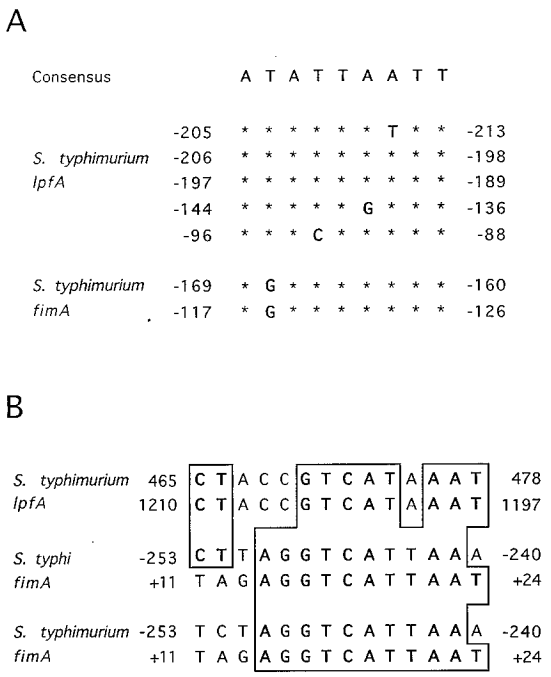

FIG. 8. (A) Comparison of repeats found in the promoter regions of *S. typhimurium lpfA* and *fimA*. The numbers indicate the position of the repeat relative to the start codon (+1) of *lpfA* and *fimA*. A consensus sequence of the repeat is given at the top. Bases in the repeats which correspond to those of the consensus sequence are indicated by stars. (B) Comparison of inverted repeats found in front of *fimA* in *S. typhimurium* and in *S. typhi* and flanking *lpfA* in *S. typhimurium*. The numbers for *lpfA* indicate the position of the repeat relative to the sequence shown in Fig. 4. The numbers for *fimA* indicate the position of the repeat relative to the start codon (+1). Conserved nucleotides are boxed.

were polar (Fig. 10) and between 2 and 10 μm long. Sometimes, fimbriae were detected at only one pole of the cell. Occasionally, more than one filament was inserted at the pole of a bacterial cell. About two-thirds of the bacteria examined displayed no fimbriae on their surfaces. Because of their morphology, these fimbriae were termed LP fimbriae.

DISCUSSION

We have identified a new fimbrial operon consisting of five genes, *lpfABCDE*, located at 78 min on the *S. typhimurium* chromosome. Expression of LP fimbriae could be detected by electron microscopy when the *S. typhimurium* operon was introduced into nonfimbriated *E. coli* ORN172. Six distinct fimbriae in *S. typhimurium* and in *S. enteritidis* have so far been described, and one in *S. typhi* has been described (Table 2). However, nucleotide sequence analysis showed that the *lpf* operon is distinct from other *Salmonella* operons for which sequence information is available, including *pef*, *fim*, *sef*, and *agf*. In addition, their polar distribution suggested that LP fimbriae are distinct from previously described *Salmonella* type 1, type 3, and GVVPQ fimbriae, all of which originate peritrichously from the bacterial surface (Table 2). In fact, LP fimbriae morphologically resemble type 4 fimbriae from other members of the *Enterobacteriaceae* in length, width, and polar distribution (28). However, there is so far no direct evidence that LP fimbriae are coded for by the *lpf* operon. Although unlikely, it is possible that the introduction of the *lpf* operon induces a cryptic fimbrial operon of *E. coli*. A comparison of the primary structures of Lpf proteins indicated that the *lpf* operon is more closely related to the *fim* operon encoding type 1 fimbriae of *S. typhimurium* than to operons encoding type 4 fimbriae. In fact, the *fim* and the *lpf* operons share the same gene order (55, 56).

An unusual feature of the *lpf* gene cluster is the lack of genes encoding regulatory proteins. In the *fim*, *pap*, and *mrp* gene clusters, one or more regulatory genes transcribed in opposite orientations are located upstream or downstream of the fimbrial operon (6, 7, 55). In the *fim* operon, these regulatory genes are thought to mediate the inversion of the *fimA* promoter, which is flanked by a 10-bp inverted repeat. The sequence of this inverted repeat is conserved in *S. typhimurium* and *S. typhi* (46, 47). A similar inverted repeat was found in the *lpf* operon, but instead of flanking the promoter of the *lpf* operon, the repeats were found up- and downstream of *lpfA*, the first gene in the operon (Fig. 8B). Thus, the positions of these repeats suggest that phase variation of the LP fimbriae may be mediated by inversion of the *lpfA* gene. The absence of regulatory genes implies that if inversion of *lpfA* occurs, it must be mediated by proteins coded for at a different locus. The similarity between the inverted repeats found in the *fim* operon and those of the *lpf* operon suggests that the latter may utilize the inversion machinery of type 1 fimbriae. In order to substantiate regulation of the *lpf* operon by phase variation, however, further experiments are necessary.

The presence of *lpf*-related sequences is restricted to *S. choleraesuis* serotypes belonging to *S. choleraesuis* subsp. *choleraesuis* (for current nomenclature on *Salmonella* spp., see reference 31). However, since only a limited number of strains was tested, we cannot exclude the possibility that *lpf*-related sequences are distributed more widely among enterobacteria. Two other fimbrial systems seem also to be restricted to *Salmonella* spp. Plasmid-encoded fimbriae are encoded by the *S. typhimurium* virulence plasmid (26). Related virulence plasmids are present in 11 other serotypes of *S. choleraesuis* subsp. *choleraesuis* but not in *S. typhi*, indicating that *pef* and *lpf* operons show similar distributions (10, 60). Type 1 fimbriae are coded for by a third fimbrial operon conserved only among *Salmonella* serotypes, as has been shown by hybridization (11, 15, 16, 56). Although the mannose-binding minor subunits of enterobacterial type 1 fimbriae are serologically conserved (1), the deduced amino acid sequences of the proteins forming this *Salmonella* adhesin have been shown to be distinct from those of type 1 fimbriae of other members of the *Enterobacteriaceae* (40, 46, 55). In addition, type 1 fimbriae are coded for by genes located at 13 and 98 min on the chromosomes of *E. coli* and *S. typhimurium*, respectively, indicating that *Salmonella* type 1 adhesins were acquired and evolved independently from those present in other genera of the *Enterobacteriaceae*. In contrast to these *Salmonella*-specific adhesin systems, several other *Salmonella* fimbrial systems were found to be distributed more widely among members of the *Enterobacteriacea*. DNA probes from the *agf* operon of *S. enteritidis* have been used to find

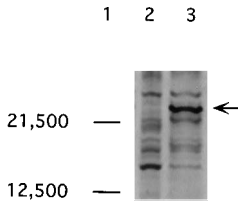

FIG. 9. Tricine-SDS-polyacrylamide gel electrophoresis of fimbrial crude extracts from *E. coli* ORN172 (lane 2) and ORN172(pMS1000) (lane 3). The positions of molecular weight standard proteins are given in lane 1. The position of a fimbrial protein in the crude extract of ORN172(pMS1000) is indicated by the arrow.

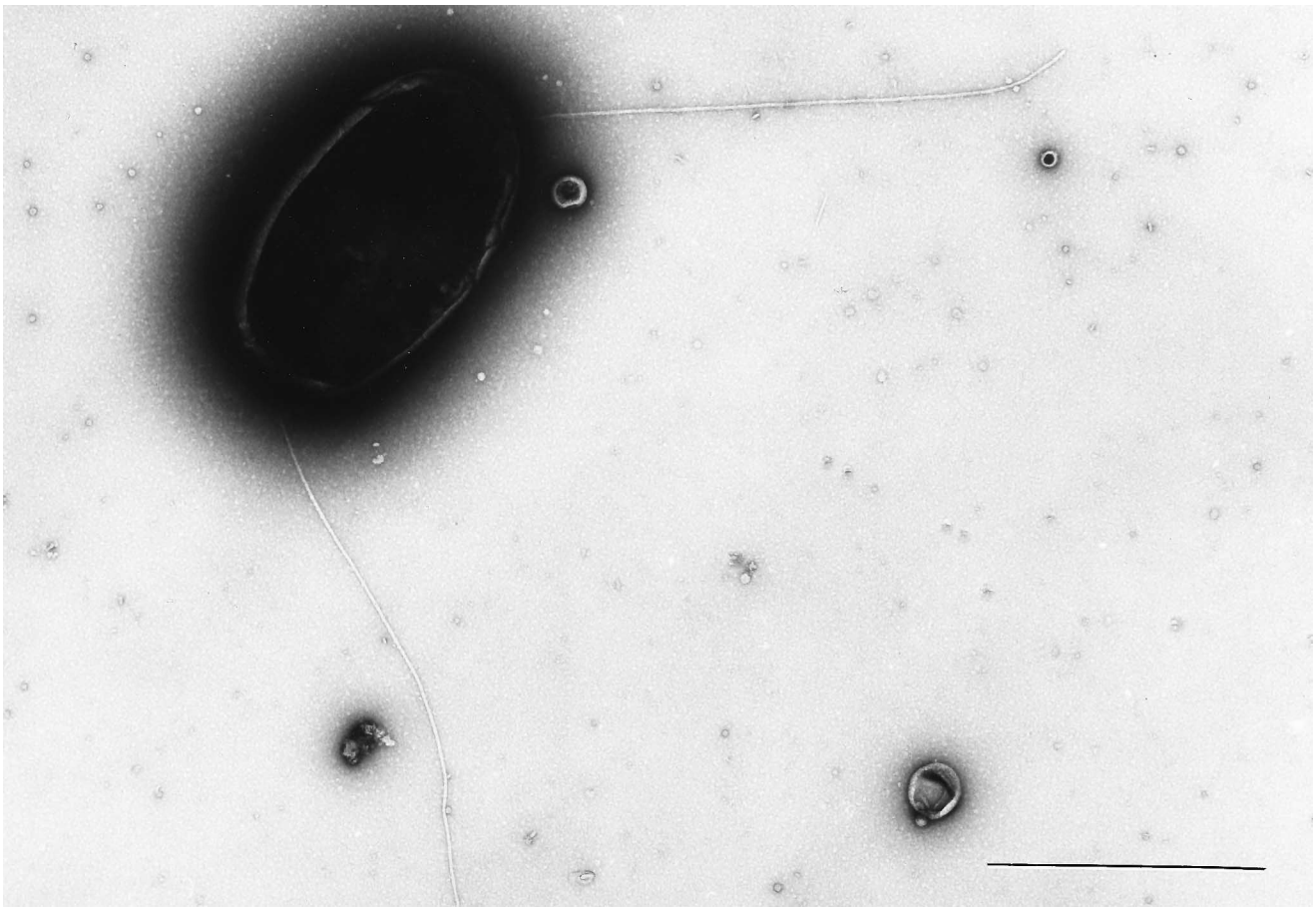

FIG. 10. Electron micrograph of *E. coli* ORN172(pMS1000). Bar, 1 μm. Final magnification, ×36,700.

related sequences not only in most *Salmonella* serotypes but also in most *E. coli* strains and in some *Shigella*, *Enterobacter*, and *Citrobacter* isolates (21). Similarly, sequences related to genes encoding bundle-forming pili of enteropathogenic *E. coli* were found in most *Salmonella* serotypes (53). Sequences related to the *sefD* gene of *S. enteritidis* were even more widely distributed among members of the *Enterobacteriaceae*, with homologs being detected in *Salmonella*, *Citrobacter*, *Entero-*

TABLE 2. Fimbrial systems of *S. typhimurium*, *S. enteritidis*, and *S. typhi*

Salmonella serovar	Fimbrial form	Fimbrial class	Distribution	Width (nm)	Genetic location (min)	Fimbrin gene	Mol wt of fimbrin	Reference(s)
S. typhimurium	LP fimbriae[a]	ND[b]	Polar[a]	7–8[a]	Chromosome (78)	ND	ND	This study
S. typhimurium	E4	Type 1	ND	ND	ND	ND	17	27
S. typhimurium	F1	Type 1	Peritrichous	7–8	Chromosome (98)	*fimA*	21	13, 46
S. typhimurium	Type 3 fimbriae	Type 3	Peritrichous	4–5	ND	ND	22	2, 23
S. typhimurium	Bundle-forming pili	Type 4	Polar	7	ND	ND	ND	53
S. typhimurium	Thin aggregative fimbriae	GVVPQ	Peritrichous	3	ND	ND	17	21, 29
S. typhimurium	Plasmid-encoded fimbriae	ND	Peritrichous	ND	Plasmid	*pefA*	15	26, 32
S. enteritidis	SEF 21	Type 1	Peritrichous	7–8	ND	*fimA*	21	40
S. enteritidis	Type 3 fimbriae	Type 3	Peritrichous	4–5	ND	ND	22	5, 23
S. enteritidis	SEF 14	Type 3-like	Peritrichous	ND	Chromosome	*sefA*	14	18, 25
S. enteritidis	SEF 18	Type 3-like	Peritrichous	Thin	Chromosome	*sefD*	18	17
S. enteritidis	Bundle-forming pili	Type 4	Polar	7	ND	ND	ND	53
S. enteritidis	SEF 17	GVVPQ	Peritrichous	3–4	ND	*agfA*	17	19
S. typhi	Type 1 fimbriae	Type 1	Peritrichous	7–8	Chromosome	*fimA*	16	47

[a] When expressed in *E. coli.*
[b] ND, no data.

bacter, *Erwinia*, *Escherichia*, *Shigella*, *Hafnia*, *Klebsiella*, *Proteus*, and *Providencia* spp. (17).

So far, only the role of type 1 fimbriae in the colonization of host niches has been studied for *Salmonella* spp. The lack of these adhesins was found to have no effect on the colonization of *S. typhimurium* in mice (37). Interestingly, type 1 fimbriae appear to be the only fimbrial system present in *S. typhi* (23, 30). This is also consistent with our finding that *lpf*-related sequences are absent from *S. typhi*. The lack of colonization factors other than type 1 fimbriae in *S. typhi* may reflect the adaptation of this pathogen to humans. The adaptation to a single mammalian host may have abolished the need for multiple fimbrial systems. In fact, type 1 fimbriae of *S. typhi* have been shown to mediate binding to human epithelial cells in vitro (50), supporting a role for these adhesins in the colonization of human host cell surfaces. In contrast to *S. typhi*, most other *S. choleraesuis* serotypes have a wide host range, including various species of livestock and wild animals. The large number of fimbrial systems described for these serotypes may thus reflect a genetic flexibility which is a prerequisite for the ability to colonize such a variety of hosts (Table 2).

The distribution of *lpf*-related sequences among *Salmonella* and related species can be explained by two possible evolutionary scenarios. One possibility is that the *lpf* operon is a recent acquisition of *S. choleraesuis* subsp. *choleraesuis* which occurred after the separation of the lineage into mouse pathogenic serotypes and *S. typhi*. Alternatively, a common ancestor of all *S. choleraesuis* subsp. *choleraesuis* serotypes may have possessed the *lpf* operon, but the operon was subsequently lost from *S. typhi* during its adaptation to its human host. Short inverted repeats flanking the *lpf* operon may be remnants of the ancient recombination event which led to the acquisition of this operon.

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