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The *fliA* Gene Encoding σ^{28} in *Yersinia enterocolitica*

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Yersinia enterocolitica **is an enterobacterium responsible for gastrointestinal syndromes. Its pathogenicity depends on the presence of the 70-kb pYV plasmid, which directs Yop secretion. The Yop secretion machinery, consisting of the YscA-U and LcrD proteins, presents some structural similarity with the flagellum assembly machinery characterized in other bacteria. Flagellum assembly requires σ^{28}, an alternative sigma factor. The region upstream of the *lcrD* gene resembles promoters recognized by σ^{28}, suggesting that the similarity between Yop secretion and flagellum assembly could extend to their regulation. The chromosome of *Y. enterocolitica* also contains pathogenicity determinants such as *myfA*, which encodes the Myf antigen subunit. The promoter region of *myfA* also resembles promoters recognized by σ^{28}. In an attempt to clarify the role of σ^{28} in the expression of *lcrD*, *myfA*, and flagellar genes, we cloned, sequenced, and mutagenized the *fliA* gene encoding the σ^{28} homolog in *Y. enterocolitica*. As is the case in other bacteria, *fliA* was required for motility. However, it was involved neither in fibrilla synthesis nor in Yop secretion. The *fliA* mutant allowed us to monitor the role of motility in pathogenesis. At least in the mouse model, motility seemed not to be required for *Y. enterocolitica* pathogenesis.**

Yersinia enterocolitica is an enterobacterium responsible for gastrointestinal diseases in humans and animals (11). Full virulence of *Y. enterocolitica* depends on the 70-kb pYV plasmid directing synthesis of adhesin YadA and Yop secretion. The machinery involved in Yop secretion is the archetype of the so-called type III secretion pathway. Many of its components have homolog not only in other animal pathogens but also in plant pathogens and in flagellum assembly mechanisms (for a review, see reference 9). For instance, LcrD of the Yop secretion machinery (42, 51) is the homolog of InvA, a protein required for invasion of *Salmonella typhimurium* (17), of MxiA, a protein involved in secretion of Ipas in *Shigella flexneri* (3), and of HrpO and HrpC2 from *Pseudomonas solanacearum* and *Xanthomonas campestris*, respectively (15, 21). Homologs of LcrD involved in flagellar assembly include FlbF of *Caulobacter crescentus* (44, 47), FlbA of *Campylobacter jejuni* (38), FlhA of *Bacillus subtilis* (8), and FlhA of *S. typhimurium* (52). Some genes encoding proteins of this family, including *lcrD*, are preceded by a sequence resembling σ^{28} promoters (3, 42, 47).

So far, the alternative sigma factor σ^{28}, also called FliA or RpoF, has been shown to be involved only in transcription of flagellar, chemotaxis, and motility genes of various enterobacteria (4, 24, 25, 31, 33, 39, 40, 50). However, the homology between the secretion machinery of *Y. enterocolitica* and the flagellum assembly machinery as well as the strong similarity between the promoter regions of *lcrD* and promoters recognized by this sigma factor suggest that a homolog of σ^{28} could be involved in Yop secretion.

The chromosome of *Y. enterocolitica* encodes an enterotoxin and a fibrillar antigen, Myf, which strongly resembles *Escherichia coli* enterotoxin-associated fimbriae (27). The promoter region of *myfA*, the gene encoding the Myf antigen subunit, contains sequences that strikingly resemble the canonical promoter recognized by the RNA polymerase σ^{28} factor (24, 26).

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Y. enterocolitica is a motile bacterium (46, 53), but it is not known whether its motility also depends on a sigma factor related to σ^{28}. It has also not been established whether motility is involved in pathogenesis.

In an attempt to clarify the role of σ^{28} in the expression of *myfA*, *lcrD*, and flagellum genes in *Y. enterocolitica*, we have cloned, sequenced, and mutagenized the *fliA* gene encoding the σ^{28} homolog of *Y. enterocolitica*. We show that *fliA* is not required for transcription of *myfA* or *lcrD*. However, *fliA* is required for motility as it is the case in many enterobacteria. At variance with other enteric pathogens, motility seems not to be involved in *Y. enterocolitica* pathogenesis.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *Y. enterocolitica* W1024 is a serotype O:9 strain. MI1024 is a *fliA*::*aphA3* mutant described in this report. *E. coli* LK111 and *E. coli* XL1-Blue were used for standard manipulations. Plasmids are listed in Table 1.

Bacteria were routinely grown in Trypticase soy broth (Oxoid, Hamphire, England) containing 0.3% (wt/vol) yeast extract and on Trypticase soy agar (Diagnostic Pasteur, Marnes la Coquette, France). For the analysis of *myfA* expression, *Y. enterocolitica* was grown on brain heart infusion (Difco, Detroit, Mich.) supplemented with 0.5% yeast extract (Gibco, BRL, Paisley, England), 2.5 mM $CaCl_2$, and 0.2% xylose (SBHI) and adjusted to pH 6 as described by Iriarte and Cornelis (26). For the induction of the *yop* regulon, *Y. enterocolitica* was grown in brain heart infusion supplemented with 4 mg of glucose ml^{-1}, 20 mM $MgCl_2$, and 20 mM sodium oxalate. The antibiotics used for selection procedures were nalidixic acid (35 $\mu g \cdot ml^{-1}$), ampicillin (200 $\mu g \cdot ml^{-1}$), streptomycin (100 $\mu g \cdot ml^{-1}$), and kanamycin (50 $\mu g \cdot ml^{-1}$).

DNA and RNA manipulations. The nucleotide sequence of *fliA* and flanking regions was determined by the method of Sanger et al. (48), using plasmids pIM123, pIM124, pIM125, pIM126, and pIM127. DNA and proteins sequences were analyzed with the Blast program (2).

RNA extraction and analysis were done as described previously (26). The probe used to detect the *myfA* transcript was prepared as described in reference 27.

Cloning of the *fliA* gene from *Y. enterocolitica*. We pooled the 760 clones of our W1024 genomic library in pLAFR3 (12) in groups of 10 and extracted cosmid DNA. DNA was digested with *Eco*RI-*Hin*dIII, separated by gel electrophoresis, transferred to membranes, and hybridized with a purified PCR fragment containing *fliA* from *S. typhimurium*. This PCR fragment was prepared as described by Starnbach and Lory (50). Cosmid pIDI43 hybridized with the probe. A 1.5-kb *Eco*RI-*Hin*dIII fragment from this cosmid was then subcloned in the corresponding sites of pBlueScript SK−, giving pSI1. To isolate the gene directly from the chromosome of *Y. enterocolitica*, *Cla*I fragments in the range of 2 to 4 kb were cloned in the corresponding site of pBlueScript KS−, and the recombinant plasmids were screened with the *Eco*RI-*Bgl*II fragment of pSI1 containing part of

TABLE 1. Plasmids used in this study

Plasmid	Characteristics	Reference
pIDI43	Member of the *Y. enterocolitica* W1024 genomic library in pLAFR3	12
pIM123	pBlueScript KS− + 2.5-kb *Cla*I fragment of *Y. enterocolitica* chromosomal DNA; contains complete *fliA* gene	This work
pIM123K	pIM123 + *aphA3* cassette in *Bgl*II site of *fliA*	This work
pIM124	pBlueScript SK− + 2.5-kb *Cla*I fragment of *Y. enterocolitica* chromosomal DNA	This work
pIM125	*Eco*RI deletion of pIM124; contains 685 bp of *fliA*	This work
pIM126	*Bgl*II-*Bam*HI deletion of pIM124; contains 325 bp of *fliA*	This work
pIM127	*Eco*RV deletion of pIM124; contains downstream region of *fliA*	This work
pIM128	pKNG101 + 3.5-kb *Sal*I-*Xba*I fragment of pIM123K; contains *fliA′*:*aphA3*:*fliA″* disrupted gene	This work
pIM130	pTM100 + 2.5-kb *Cla*I fragment of *Y. enterocolitica* chromosomal DNA; contains complete *fliA* gene	This work
pIM130K	pIM130 + *aphA3* cassette in *Bgl*II site of *fliA* gene	This work
pKNG101	*pir-ori*$_{R6K}$ *ori*$_{RK2}$ *strAB sacBR*	29
pSI1	pBlueScript SK− + *Eco*RI-*Hin*dIII fragment of pIDI43; contains 366 bp of *fliA* from *Y. enterocolitica*	This work
pTM100	pACYC184 + *oriT*$_{RK2}$ cloned in the *Sst*II site (coordinate 832 bp)	36
pUC18K	850-bp *aphA3* cassette inserted into the *Sma*I site of plasmid pUC18	35

Y. enterocolitica fliA. Clone pIM123 contained the 2.5-kb *Cla*I fragment enclosing the complete *fliA* gene.

Construction of a *Y. enterocolitica* W1024 *fliA* mutant. First, plasmid pIM123 containing the complete *fliA* gene was cleaved at a *Bgl*II site situated 367 bp downstream from the ATG start codon of *fliA*, and the ends were filled with Klenow enzyme. The *aphA3* cassette (35) was extracted from its carrying plasmid, pUC18K, with enzymes *Eco*RI and *Bam*HI. Both ends were filled with Klenow enzyme. Insertion of the *aphA3* cassette in the filled *Bgl*II site of *fliA* gave pIM123K. In this plasmid, the *aphA3* cassette is flanked by 367 bp of *fliA* on the 5′ side and 324 bp of *fliA* on the 3′ side. Next, we transferred the *Sal*I-*Xba*I fragment of pIM123K containing the *fliA′-aphA3-fliA″* construct into the corresponding sites of the suicide vector pKNG101 containing a streptomycin resistance gene and the *sacB* gene encoding levanesucrase and conferring sensitivity to sucrose (29). The resulting mutator plasmid, pIM128, was mobilized in *Y. enterocolitica* W1024 by conjugation, and the chromosomal *fliA* gene was replaced by the *fliA′-aphA3-fliA″* construct by double recombination. The selection for the *fliA* mutant was based on resistance to kanamycin and sucrose and on sensitivity to streptomycin. Allelic exchange was finally checked by Southern hybridization.

Protein analysis. The Yops were prepared and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Cornelis et al. (10).

Motility assay. Bacteria were inoculated with a pipette into a local point of semisolid plates containing 0.3% agar. The capacity of each strain to spread beyond the inoculation point was monitored after 24 h of incubation at 22°C.

Virulence assay. To study the virulence of the *fliA* mutant, we inoculated

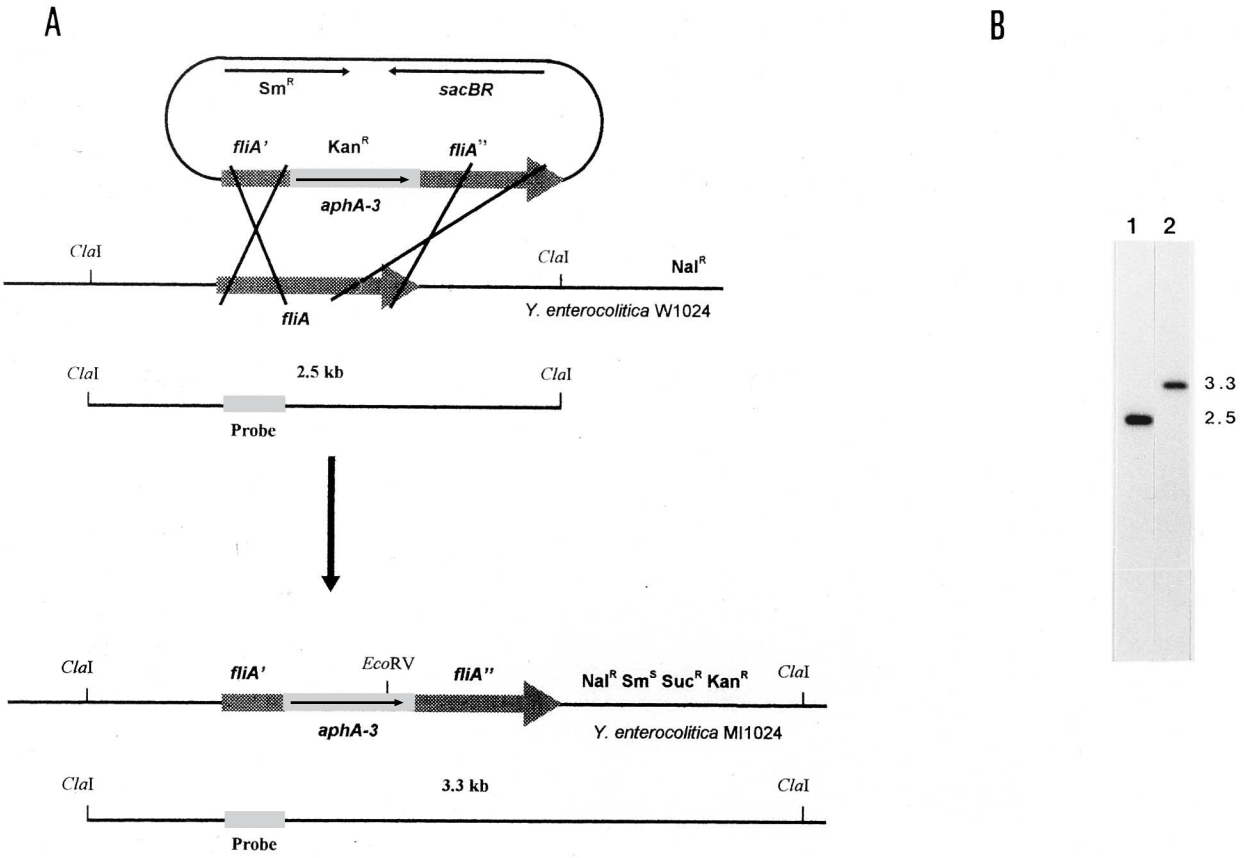

FIG. 1. (A) Construction of the *fliA* (=rpoF) mutant by double recombination using plasmid pIM123K. (B) Southern hybridization analysis of the wild-type strain W1024 (lane 1) and the *fliA* mutant MI1024 (lane 2). Chromosomal DNA was digested with *Cla*I. Sizes are indicated in kilobases.

8-week-old female BALB/c mice by gastric intubation with 300 μl of a suspension containing 10^{10} bacteria of either the wild-type strain W1024 or the *fliA* mutant MI1024 and 2.5 mg of Desferal (CIBA-GEIGY, Brussels, Belgium). Mice were killed at 18 h, 2 days, and 7 days postinfection. For each mouse, three Peyer's patches were excised from the ileum, washed in 0.7% NaCl–0.1% Triton X-100, and homogenized together in 5 ml of phosphate-buffered saline (PBS; 50 mM sodium phosphate, 150 mM NaCl [pH 7.4]) as described by Sory et al. (49). Appropriate dilutions of the homogenized tissues were immediately plated on Trypticase soy agar containing nalidixic acid (wild type) or nalidixic acid and kanamycin (*fliA* mutant). The spleens were harvested only at days 2 and 7 postinfection, resuspended in 5 ml of PBS, homogenized, and plated as described for Peyer's patches.

Nucleotide sequence accession number. The sequence of *fliA* and flanking regions has been submitted to GenBank under accession number U17393.

RESULTS

Cloning and sequencing of the *fliA* gene from *Y. enterocolitica*. To isolate the *fliA* gene, we screened the cosmids from our genomic library of *Y. enterocolitica* W1024 (12) by using as a probe PCR-amplified *fliA* from *S. typhimurium* (39). Cosmid pIDI43, hybridizing with this probe, was further analyzed by Southern blotting, and a 1.5-kb *Eco*RI-*Hin*dIII fragment was subcloned in pBlueScript SK−, giving pSI1. Sequence analysis revealed that pSI1 and pIDI43 contained only the 5′ end (366 bp) of the *fliA* gene. This partial *fliA* sequence from *Y. enterocolitica* was in turn used as a probe to localize the *fliA* gene in digests of chromosomal DNA of *Y. enterocolitica* W1024. This Southern blot analysis showed that the *fliA* gene was contained within a 2.5-kb *Cla*I fragment. This fragment was cloned, giving pIM123, and 1.4 kb were sequenced. The *fliA* gene starts at position 493 and ends at position 1185. It is preceded by a putative Shine-Dalgarno sequence (GAAGG) located 9 bp upstream from the ATG start codon. It encodes a protein of 230 amino acid residues with a predicted molecular mass of 26.5 kDa. The deduced amino acid sequence is 83% identical to FliA of *S. typhimurium* (39), 36.6% identical to σ^{28} of *B. subtilis* (25), and 52.7% identical to FliA of *Pseudomonas aeruginosa* (50).

Upstream from *fliA*, we identified the coding region for the carboxy-terminal end (90 amino acid residues) of a protein with 35% identity to the FliV protein involved in flagellum assembly in *Salmonella* spp. (13). In *Y. enterocolitica*, the *fliV* stop codon is separated by 220 bp from the *fliA* start codon. No palindromic sequence could be identified between these two genes, which have the same transcription polarity.

Downstream from *fliA* and in the same orientation, we identified the coding region for the amino-terminal part of a putative protein (open reading frame X), similar to an open reading frame situated downstream from *fliA* in the *S. typhimurium* chromosome and whose function has not been established (data not shown).

Construction of a *fliA Y. enterocolitica* mutant. To determine the role of *fliA* in the expression of virulence factors, we constructed a *Y. enterocolitica fliA* mutant by allelic exchange (see Materials and Methods). To generate a nonpolar mutation, we used the *aphA3* cassette described by Ménard et al. (35). This cassette, which contains a start codon in the 3′ region was cloned in such a way as to allow translation of the remaining 3′ portion of *fliA* and possible downstream genes (Fig. 1). The W1024 *fliA* mutant was called MI1024.

Influence of the *fliA* mutation in motility. In *E. coli*, *S. typhimurium*, and *B. subtilis*, the σ^{28} factor is involved in expression of motility, chemotaxis, and flagellar genes (for a review, see reference 24). We thus tested the motility of the wild-type strain *Y. enterocolitica* W1024 and of the *fliA* mutant MI1024. As can be seen in Fig. 2, the wild-type strain was motile while the *fliA* mutant was not. To demonstrate that mutation of *fliA* was responsible for the nonmotility of strain

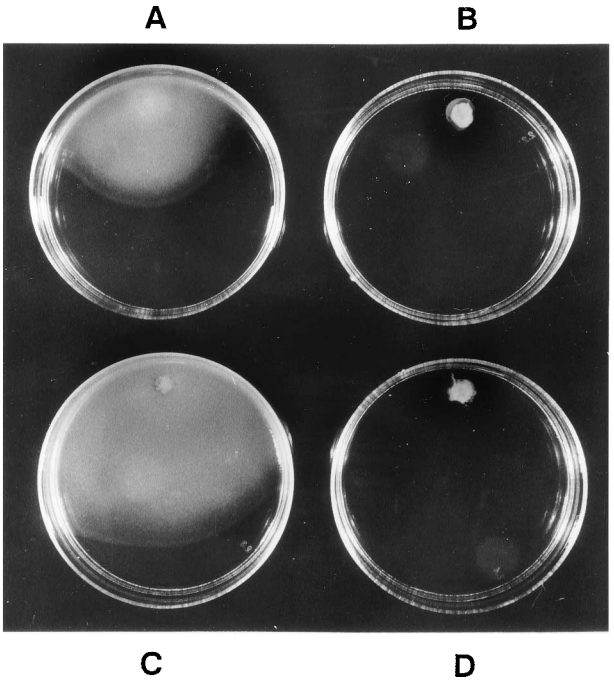

FIG. 2. Influence of the *fliA* mutation on motility. (A) W1024 (wild-type strain); (B) MI1024 (*fliA* mutant); (C) MI1024(pIM130); (D) MI1024 (pIM130K).

MI1024, we complemented the mutation with a cloned *fliA* gene. Therefore, the 2.5-kb *Cla*I fragment of chromosomal DNA containing *fliA* was subcloned in pTM100, a medium-copy-number mobilizable plasmid derived from pACYC184, to obtain pIM130. We also subcloned the cassette-inactivated *fliA* gene into the same vector to obtain pIM130K. As can be seen

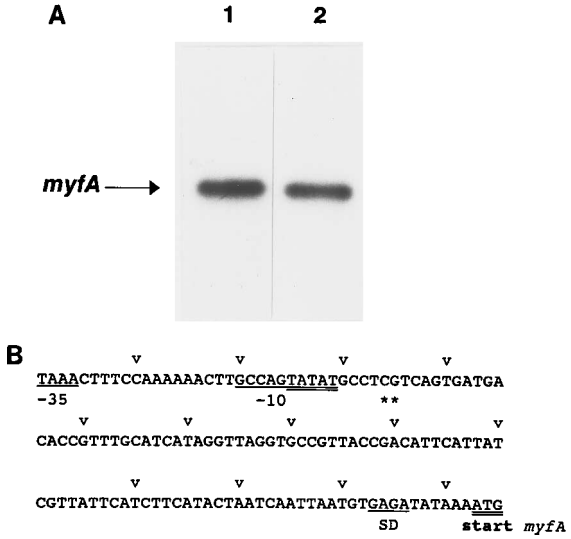

FIG. 3. (A) Influence of *fliA* mutation on transcription of *myfA*. Total RNA was extracted from bacteria grown at 37°C in SBHI adjusted to pH 6. Transcripts were detected with a PCR probe corresponding to the central part of *myfA*. Lanes: 1, W1024 (wild type); 2, MI1024 (*fliA* mutant). (B) Nucleotide sequence of the region upstream from *myfA*. The transcription start of *myfA* is shown by two asterisks. Putative −10 and −35 boxes for a σ^{28} promoter are underlined once. The −10 box for the vegetative sigma factor is underlined twice. The putative ribosome binding site for *myfA* is designated SD (from reference 26).

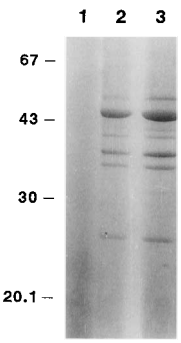

FIG. 4. Influence of *fliA* mutation on Yop production. Lanes: 1, W1024(pYV−); 2, W1024(pYV+); 3, MI1024 (pYV+). Sizes are indicated in kilodaltons.

in Fig. 2, motility was restored to normal levels when pIM130 was introduced in the *fliA* mutant. However, the mutation was not complemented by plasmid pIM130K. Hence, σ^{28} is involved in motility of *Y. enterocolitica.*

Influence of *fliA* mutation on transcription of *myfA*. To analyze the influence of the *fliA* mutation on Myf production, we monitored the production of Myf by SDS-PAGE. We did not observe any difference between W1024 and MI1024 (data not shown). To confirm this result, we also analyzed *myfA* transcription. Total RNA was extracted from bacteria grown on SBHI adjusted to pH 6 (26) and analyzed by Northern (RNA) blotting using a *myfA*-specific probe (27). Again, we could not detect any difference in the expression of *myfA* between the wild type and the *fliA* mutant (Fig. 3), showing that σ^{28} is not required for *myfA* expression.

Influence of *fliA* mutation in Yop production. We then studied the role of FliA in expression of *lcrD*, a gene encoding an inner membrane protein of the Yop secretion machinery. If transcription of *lcrD* requires the σ^{28} RNA polymerase, the *fliA* mutant of *Y. enterocolitica* should be unable to secrete the Yops. We thus tested the secretion of Yops by the *fliA* mutant. The *fliA* mutant and its wild-type parental strain released the same amount of Yops (Fig. 4). Hence, *fliA* is not required for *lcrD* transcription.

Role of motility on invasion capacity of *Y. enterocolitica* in vivo. We used our *fliA* mutant to determine the role of the alternative σ^{28} factor in pathogenicity. We infected intragastrically two groups of 12 mice with 10^{10} bacteria of the wild-type strain or the *fliA* mutant and determined the number of bacteria in Peyer' patches and spleen after 18 h, 2 days, and 7 days. The mutation was stable after passage in vivo. The results (Table 2) did not show any difference in the invasion capacity of the mutant and the wild-type strain. Hence, no σ^{28}-dependent genes are required for pathogenicity of *Y. enterocolitica* in the mouse model. Motility is thus not required for invasion.

DISCUSSION

Genes involved in flagellum expression are tightly regulated and organized in a complex hierarchy. In *E. coli* and *S. typhimurium*, the first level of this cascade includes the *flhDC* operon, which controls expression of class II genes. Class II genes encode proteins forming the basal body hook structure, and also the FliA protein (also called σ^{28} or RpoF), an alternative sigma factor of the RNA polymerase that is required for transcription of class III genes. This last level of the hierarchy includes flagellin genes as well as genes involved in motility and chemotaxis (for reviews, see references 24 and 34). The activity of FliA is negatively regulated by FlgM, which acts as an anti-sigma factor, binding to FliA and preventing its association with the RNA polymerase core (32). In a recent work, Kutsukake and Iino (31) presented evidence suggesting that FliA may also act as an activator in the transcription of class II flagellar operons. Genes *flhB*, *-A*, and *-E* as well as *flgN*, *-M*, *-A*, *-B*, *-C* and, *-D* have recently been identified and sequenced in *Y. enterocolitica* W1024 (14). In this work we identified, cloned, sequenced, and mutagenized a homolog of *fliA* in the same *Y. enterocolitica* strain. The *fliA* gene has been identified on a cosmid different from the one containing *flh* and *flg*. Hence, these genes are presumably not linked, suggesting that the flagellar genes are scattered in various regions of the chromosome as described for other bacterial species. The FliA protein has 83% identity with its *S. typhimurium* counterpart (39) and, like the latter, is involved in motility.

The proteins that constitute the Yop secretion machinery show significant homology to proteins involved in flagellum assembly, more specifically to products of class II genes of the flagellar regulon cascade (1, 18, 43, 54). Interestingly, upstream from *lcrD* there is a sequence that matches closely the consensus for the flagellar sigma factor (Table 3). It has therefore been suggested that production of inner membrane protein LcrD could involve this alternative sigma factor (47). However, we did not observe any difference in Yop secretion between the wild-type strain and the *fliA* mutant. Thus, although the promoter region of *lcrD* strongly resembles promoters recognized

TABLE 2. Bacterial counts in Peyer's patches and spleens after intragastric inoculation of W1024 (*fliA*$^+$) and MI1024 (*fliA*) to mice[a]

Strain	No. of bacteria recovered				
	Per Peyer's patch			Per spleen	
	18 h (10^5)	2 days (10^6)	7 days (10^5)	2 days (10^2)	7 days (10^3)
W1024	1.5	2.6	1.7	1	0
	3.1	1.6	2.1	85	0
	1.5	1.0	3.4	2	0
	1.0	1.9	2.3	0	0
Mean ± SE	1.8 ± 0.8	1.8 ± 0.6	2.4 ± 0.6	22 ± 36.4	
MI1024	1.1	2.2	2.7	0.3	0
	0.5	1.3	2.7	3.0	1.3
	0.7	3.0	1.7	2.0	0
	1.5	2.5	1.1	9.0	0
Mean ± SE	1.0 ± 0.4	2.3 ± 0.6	2.1 ± 0.7	3.6 ± 3.3	

[a] The Peyer's patches and spleen were excised after the indicated time. Four mice were sacrified at each time point.

TABLE 3. Promoter sequence and role of σ^{28}

Promoter	Sequence[a]	Transcription by σ^{28}	Reference
Consensus σ^{28}			
B. subtilis	$TAAAN_{15}GCCGATATN_{7-10}$ +1	+	19, 20
E. coli	$TAAAN_{15}GCCGATAAN_{7-10}$ +1	+	4
S. typhimurium	$TAAAN_{15}GCCGATAAN_{7-10}$ +1	+	33
Y. enterocolitica myfA	$TAAAN_{15}GCCAGTATN_{7}$ +1	−	26, this work
Y. enterocolitica lcrD	$TAATN_{15}GCCGAAAA$	−	47
Shigella flexneri mxiA	$TTAAN_{15}GCCTGAAT$	NT[b]	3

[a] Underlined nucleotides deviate from the consensus.
[b] NT, not tested.

by σ^{28} and despite the similarity between the Yop secretion machinery and the flagellum assembly machinery, the two systems are regulated by different sigma factors. This result fits with the observations that motility and Yop production are expressed under different temperature conditions. It is indeed consistent that these two related systems are not expressed at the same time at the bacterial surface, because the simultaneous production of similar proteins could lead to misassembly of both machineries. Finally, considering the known promoter sequence of *mxiA* (3) and our result concerning *lcrD*, we would like to predict that transcription of *mxiA*, the *lcrD* homolog in *Shigella flexneri*, is probably independent of σ^{28}.

The genes encoding the Myf fibrillae are also preceded by a sequence resembling a typical σ^{28}-dependent promoter and located 7 or 8 nucleotides upstream from the transcription start, mapped by primer extension (Fig. 3 and Table 3) (26). We show in this work that σ^{28} is not required for transcription of *myfA*. The only difference between the −10 box of the *myfA* promoter and that of the consensus σ^{28} promoter is an inversion of two nucleotides (AG for *myfA* instead of GA), indicating that σ^{28} tolerates little deviation from its consensus promoter sequence. The *myfA* gene could also be transcribed by the vegetative RNA polymerase. A putative −10 box similar to that recognized by the vegetative sigma factor was indeed identified upstream from the transcription start of *myfA*, but no sequence similar to the −35 box was detected upstream from this −10 box. The absence of −35 box is usual among genes which require a transcriptional activator, and this is indeed the case of *myfA*, which is positively regulated by *myfF* (26). In conclusion, although the promoters of *lcrD* and *myfA* strongly resemble the consensus promoter of σ^{28}, they are not recognized by this factor. These observations are summarized in Table 3.

In order to colonize the intestinal tract, bacteria should be able to penetrate deep within the mucus in order to reach the surface of the intestinal epithelium. The presence of flagella at the bacterial surface could facilitate migration through the mucus and further colonization of the intestinal epithelium, but this remains a matter of debate. Jones et al. (28) have shown that the ability of a nonmotile *S. typhimurium* isolate to enter Peyer's patches in vivo is reduced 10-fold compared with that of the wild type. In agreement with this observation, motility seems to be required for *S. typhimurium* invasion of epithelial cells in vitro (6, 30). In *C. jejuni*, flagella also play a role in penetration of cultured cells of human intestinal epithelial origin, but they are not involved in adhesion to epithelial cells (22). Studies with human volunteers have confirmed this role of flagella in pathogenesis by showing that there is a selection for flagellated *C. jejuni* in the intestinal tract (7). Finally, motility and chemotaxis are also important virulence factors of *Vibrio cholerae*. In this organism, flagella allow bacteria to move, and at the same time they can act as an adhesin. Nonmotile mutants are less virulent, exhibit reduced adherence to isolated rabbit brush borders, and provoke significantly less fluid accumulation than their wild-type parental strains (5, 16, 23, 45). In *Y. enterocolitica*, the fact that motility is detected in vitro only when bacteria are grown at 28°C could be taken as an argument against a possible role of flagella in pathogenesis. However, downregulation of flagellar genes by temperature could be modified by other environmental factors prevailing in the human intestine, such as pH and osmolarity, as is the case for the enterotoxin Yst (37) or the invasin Inv (41). To assess the role of motility in virulence of *Y. enterocolitica*, we infected mice intragastrically with the wild-type strain and the *fliA* nonmotile mutant. We did not observe any difference in the capacity of both bacteria to invade the Peyer's patches and the spleen even though the mutation was stable after passage in vivo. These results suggest that at least in the mice model, pathogenic *Y. enterocolitica* does not need flagella to penetrate the mucus layer and to further invade the target organs.

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