Role of Motility and the *flhDC* Operon in *Escherichia coli* MG1655 Colonization of the Mouse Intestine[▽]

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Previously, we reported that the mouse intestine selected mutants of *Escherichia coli* MG1655 that have improved colonizing ability (M. P. Leatham et al., Infect. Immun. 73:8039–8049, 2005). These mutants grew 10 to 20% faster than their parent in mouse cecal mucus in vitro and 15 to 30% faster on several sugars found in the mouse intestine. The mutants were nonmotile and had deletions of various lengths beginning immediately downstream of an *IS1* element located within the regulatory region of the *flhDC* operon, which encodes the master regulator of flagellum biosynthesis, FlhD₄C₂. Here we show that during intestinal colonization by wild-type *E. coli* strain MG1655, 45 to 50% of the cells became nonmotile by day 3 after feeding of the strain to mice and between 80 and 90% of the cells were nonmotile by day 15 after feeding. Ten nonmotile mutants isolated from mice were sequenced, and all were found to have *flhDC* deletions of various lengths. Despite this strong selection, 10 to 20% of the *E. coli* MG1655 cells remained motile over a 15-day period, suggesting that there is an as-yet-undefined intestinal niche in which motility is an advantage. The deletions appear to be selected in the intestine for two reasons. First, genes unrelated to motility that are normally either directly or indirectly repressed by FlhD₄C₂ but can contribute to maximum colonizing ability are released from repression. Second, energy normally used to synthesize flagella and turn the flagellar motor is redirected to growth.

Intestinal colonization is defined as the indefinite persistence of a bacterial population in the intestine of an animal in stable numbers without repeated introduction of the bacterium into the animal. Commensal *Escherichia coli* strains colonize the mouse intestine by growing in the intestinal mucus layer which covers the epithelium (18, 24, 25, 28, 38, 39, 43). The intestinal mucus layer is constantly being synthesized, degraded by indigenous bacteria, and sloughed into feces (15, 27). Colonization requires bacteria to penetrate the mucus layer, compete for nutrients with the indigenous flora, and divide at a rate that is at least equal to the washout rate caused by sloughing of the mucus layer into feces (7, 16); however, the role of motility in mucosal colonization is complex and appears to differ in different microorganisms (12).

Motility and chemotaxis in *E. coli* are controlled by a master regulator encoded by *flhD* and *flhC*, which comprise the *flhDC* operon. Together, FlhD and FlhC form the FlhD₄C₂ complex (20, 44), which activates transcription of class II flagellar genes that encode components of the flagellar basal body and export machinery (30). One of the class II flagellar genes, *fliA*, encodes an RNA polymerase sigma factor, σ^{28} , which switches on expression of the class III genes coding for the cell-distal structural components of the flagellum (30). The sequenced *E. coli* MG1655 strain has an *IS1* element in the regulatory region of the *flhDC* promoter (3), which increases the expression of the

flhDC operon and makes this strain hypermotile compared to the isogenic strain in which this element is missing (2).

Recently, we showed that the mouse intestine selects nonmotile Δ *flhDC* mutants of *E. coli* MG1655 that originate immediately downstream of the *IS1* element (17). These mutants have improved colonizing ability and grow 10 to 20% faster than their parent in mouse cecal mucus in vitro and 15 to 30% faster on a variety of sugars as sole carbon and energy sources in vitro (17). *E. coli* MG1655 *flhDC* operon deletion mutants might utilize carbon sources better than their parent as a result of release of repression of genes normally regulated by the FlhD₄C₂ regulatory complex (e.g., the complex is known to repress *gluA* [citrate synthase], *sdhCDAB* [succinate dehydrogenase], *mdh* [malate dehydrogenase], and *mglBAC* [galactose transport] [31, 32]) or as a result of increased energy that is available for other cellular processes in the absence of hyperflagellum synthesis and rotation. In the present study, these possibilities were examined.

MATERIALS AND METHODS

Bacterial strains. *E. coli* MG1655 Str^r is a spontaneous streptomycin-resistant mutant of the sequenced wild-type *E. coli* MG1655 strain (CGSC 7740) (24). It has an *IS1* element in the *flhDC* promoter (3). *E. coli* MG1655 Str^r Nal^r is a spontaneous nalidixic acid-resistant mutant of *E. coli* MG1655 Str^r (24). It is referred to below as *E. coli* MG1655 (+*IS1*) (hypermotile, hyper-*flhDC* expression). Its *flhDC* locus is illustrated in Fig. 1. *E. coli* MG1655 (CGSC 6300) was obtained from the *E. coli* Genetic Stock Center and is a wild-type strain that lacks the *IS1* element in the *flhDC* promoter. *E. coli* MG1655 (–*IS1*) Str^r Nal^r, constructed as described below, is isogenic with MG1655 Str^r except that it is nalidixic acid resistant and contains the *flhDC* promoter region of MG1655 (CGSC 6300). Because this strain is missing the *IS1* element in the *flhDC* promoter, it is less motile than *E. coli* MG1655 (+*IS1*) (Fig. 2); it is referred to below as *E. coli* MG1655 (–*IS1*) (normally motile, normal *flhDC* expression). Its *flhDC* locus is illustrated in Fig. 1. *E. coli* MG1655 Str^r Δ *flhD::cam* has a 546-bp

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FIG. 1. *flhDC*, *motAB*, and *fliC* loci of *E. coli* MG1655 (+IS1), *E. coli* MG1655 (–IS1), *E. coli* MG1655 (+IS1) Δ*flhD*, and *E. coli* MG1655 (+IS1) Δ*motAB* Δ*fliC*. The arrows above genes indicate promoters and directions of transcription.

deletion beginning immediately downstream of *IS1* in the regulatory region of *flhD* and continuing into *flhD*. The deletion inactivates the entire *flhDC* operon (17). *E. coli* MG1655 Str^r Δ*flhD*::*cam* is streptomycin and chloramphenicol resistant and is referred to below as *E. coli* MG1655 (+IS1) Δ*flhD* (nonmotile, no *flhDC* expression). Its *flhDC* locus is illustrated in Fig. 1. *E. coli* MG1655 Str^r Δ*motAB* Δ*fliC*::*cam* was constructed from *E. coli* MG1655 Str^r and has the *IS1* element in the *flhDC* promoter. It is both streptomycin and chloramphenicol resistant, has a 1,711-bp deletion encompassing the *motA* and *motB* genes which inactivates the flagellar motor, and has a 583-bp deletion in *fliC*, preventing flagellin synthesis. It is referred to below as *E. coli* MG1655 (+IS1) Δ*motAB* Δ*fliC* (nonmotile, hyper-*flhDC* expression) and was constructed as described below. Its *flhDC*, *motAB*, and *fliC* loci are illustrated in Fig. 1. *E. coli* MG1655 Str^r Δ*edd*::*kan*, referred to below as *E. coli* MG1655 Δ*edd*, was constructed from *E. coli* MG1655 Str^r (17). It grows poorly using gluconate as a sole carbon and energy source compared to the wild type (17), lacks 6-phosphogluconate dehydratase, and is both streptomycin and kanamycin resistant. *E. coli* MG1655 Str^r Δ*edd*::*kan*, referred to below as *E. coli* MG1655 Δ*edd**, was isolated from the feces of a mouse 20 days after feeding of MG1655 Str^r Δ*edd*::*kan* (17). It has a 2,384-bp deletion beginning immediately downstream of *IS1* in the regulatory region of *flhD* and continuing through *flhD*, *flhC*, and *motA* and into *motB* (17). *E. coli* MG1655 Δ*edd** grows better than *E. coli* MG1655 Δ*edd* on gluconate and on a number of other sugars and is a better mouse intestine colonizer than *E. coli* MG1655 Δ*edd* (17).

FIG. 2. Spread of *E. coli* MG1655 (+IS1) and *E. coli* MG1655 (–IS1) on Luria motility agar after 8 h at 37°C.

Media and growth conditions. Luria broth (LB) was made as described by Revel (34). Luria agar is LB containing 12 g of Bacto agar (Difco) per liter. MacConkey agar (Difco) was prepared according to the manufacturer's instructions. M9 minimal medium (23) was supplemented with NaCl to bring the total NaCl concentration to 200 mM and with either reagent grade glycerol (0.4%, wt/wt) or a mixture of L-arabinose, L-fucose, D-galactose, D-gluconate, N-acetyl-D-glucosamine, D-glucuronate, D-maltose, D-mannose, D-melibiose, and D-ribose, each at a concentration of 0.005% (wt/wt). Inocula were prepared as follows. Overnight cultures grown in LB were started from single colonies picked from Luria agar plates. The LB cultures were washed twice in M9 minimal medium (containing 200 mM NaCl and no carbon source), and 10-μl portions of the washed cultures were transferred to M9 minimal glycerol medium (containing 200 mM NaCl) and incubated overnight. The next morning, the cultures were washed twice as described above and were diluted to an *A*₆₀₀ of 0.05 into fresh 20-ml aliquots of M9 minimal medium (containing 200 mM NaCl) containing the 10-sugar mixture. Cultures were incubated at 37°C with shaking in 125-ml tissue culture bottles. Growth was monitored spectrophotometrically at *A*₆₀₀ using a Pharmacia Biotech Ultrospec 2000 UV/visible spectrophotometer.

Construction of *E. coli* MG1655 (–IS1). Primers used to construct deletion mutants were designed based on the MG1655 genome sequence (3). The DNA procedures used have been described previously (24). *E. coli* MG1655 (CGSC 7740) contains an *IS1* element in the promoter of the *flhDC* operon (3). *E. coli* MG1655 Str^r Δ*flhD*::*cam* has a 546-bp deletion originating immediately downstream of *IS1* in the *flhD* promoter and extending into *flhD* (Fig. 1). *E. coli* MG1655 (CGSC 6300) is missing the *IS1* element in the *flhDC* promoter (2). Using the system of Datsenko and Wanner (8), the region 121 bp upstream of the *IS1* element in CGSC 7740 and the 546-bp deletion in *flhD* were replaced with the wild-type sequence of *E. coli* MG1655 (CGSC 6300), which lacks the *IS1* element, as follows. The primers used were: forward primer, 5'-CCTGTTTCA TTTTGTCTTGCTAGC-3'; and reverse primer, 5'-GGAATGTTGCGCCTCA CCG-3'. For isolation of the desired transformant, since MG1655 (CGSC 6300) is motile and *E. coli* MG1655 Str^r Δ*flhD*::*cam* is nonmotile, 2-μl aliquots of the transformation mixture were spotted onto Luria motility agar plates (see below), which were then incubated at 37°C overnight. The next day, motile colonies were observed emanating from the site of each spot. Several colonies were picked and tested for sensitivity to chloramphenicol and ampicillin. One isolate, which was sensitive to both chloramphenicol (replacement of the deleted *flhD* gene with wild-type *flhD*) and ampicillin (loss of pKD46 [8]), was sequenced (see below) and thereby shown to be missing the *IS1* element in the *flhDC* promoter, to have the same *flhDC* promoter sequence as *E. coli* MG1655 (CGSC 6300), and to have the wild-type *flhD* gene. This strain was named *E. coli* MG1655 (–IS1) Str^r. A spontaneous nalidixic acid-resistant mutant was isolated from *E. coli* MG1655

(-IS1) Str^r after 10⁸ CFU was plated on Luria agar plates containing nalidixic acid (50 µg/ml). This strain was named *E. coli* MG1655 (-IS1) Str^r Nal^r and is referred to below as *E. coli* MG1655 (-IS1) (Fig. 1).

Construction of *E. coli* MG1655 (+IS1) Δ motAB Δ fliC. The *E. coli* MG1655 (+IS1) Δ motAB deletion mutant was constructed by allelic exchange as described by Datsenko and Warner (8). A total of 1,711 bp was deleted from *motA* (which encodes the proton conductor component of the flagellum motor) and *motB* (which encodes the protein that enables flagellar motor rotation) beginning immediately downstream of the *motA* GTG start codon and ending 96 bp upstream of the *motB* TGA stop codon. The *motAB* deletion primers (bold type indicates MG1655 DNA, and underlining indicates chloramphenicol resistance cassette DNA) were: primer 1, 5'-**TGTTCTCTCGGTACAGT**TTTCGGCGGTTAGTGTAGGCTGGAGCTGCTTCG-3'; and primer 2, 5'-**CCAGGGCGCTTACT**GGCTCATCTTGGCTTCGGCTATGAATATCCTCCTTAGT-3'. The chloramphenicol resistance cassette was then removed (8). The *E. coli* MG1655 (+IS1) Δ motAB mutant was then used to construct the *E. coli* MG1655 (+IS1) Δ motAB Δ fliC deletion mutant (Fig. 1). A total of 583 bp was deleted from *fliC* (which encodes flagellin) beginning at the ATG start codon and ending 910 bp upstream of the TAA stop codon. The *fliC* deletion primers (bold type indicates MG1655 DNA, and underlining indicates chloramphenicol resistance cassette DNA) were: primer 1, 5'-**AAACCAATACGTAATCAACGACTTGCAATATAG**GATAACGAATCGTGTAGGCTGGAGCTGCTTCG-3', and primer 2, 5'-**GGCTGCTTCGCTAGAAAGGGTAATCCAGTAAGTTTAAATATTGTTTCATATGA**ATATCCTCCTTAGT-3'.

Sequencing. DNA sequencing was done at the URI Genomics and Sequencing Center, University of Rhode Island, Kingston, using the CEQ8000 genetic analysis system (Beckman Coulter, Fullerton, CA). A Dye Terminator cycle sequencing Quick Start kit (Beckman Coulter) was used in the sequencing reactions. The primers used to amplify PCR products for sequencing to determine the precise location of the deletion in *motAB* were: forward primer (upstream of *motA*), 5'-CATCCTGTCATGGTCAACAGTGG-3'; and reverse primer (downstream of *motB*), 5'-CGTGAAGCCAAAGTTCTCTGC-3'. The same primers were used in the sequencing reactions. The primers used to amplify PCR products for sequencing to determine the precise location of the deletion in *fliC* were: forward primer (upstream of *fliC*), 5'-GGGGTTATCGGTCTGAATTGC-3'; and reverse primer (extension begins 759 bp downstream of the *fliC* coding sequence), 5'-AGTCGCCATTGTCACTGTACC-3'. These primers were also used in the *fliC* sequencing reactions. The primers used to PCR amplify the *flhDC* promoter region in MG1655 (-IS1) were the primers used above for making the strain. The primer used for sequencing was the forward primer used for PCR amplification (i.e., 5'-CCTGTTTCATTTTGTCTGCTAGC-3'), which is upstream of IS1.

Motility. Normally, Luria motility agar is LB containing 3.5 g of Bacto agar per liter. However, for measurement of motility in the presence of various concentrations of NaCl, three colonies of each strain were transferred with toothpicks to motility agar plates containing LB diluted 10-fold with distilled water and each of the NaCl concentrations. The diluted LB was used to better control the NaCl concentration. Motility agar plates were incubated for 5.5 h at 37°C, and then spreading was measured. For testing the motility of fecal isolates from mice, 50 colonies from each mouse on each day of sampling were transferred with toothpicks to motility agar made from LB that had been diluted 10-fold with distilled water but contained no added NaCl. The preparations were incubated overnight at room temperature. Under these conditions, it was possible to distinguish motile colonies from nonmotile colonies on a single plate inoculated with 50 colonies.

Mouse colonization experiments. The method used to compare the large intestine-colonizing abilities of *E. coli* strains in mice has been described previously (38, 39, 43). Briefly, three male CD-1 mice (5 to 8 weeks old) were given drinking water containing streptomycin sulfate (5 g/liter) for 24 h to eliminate the resident facultative bacteria (22). Following 18 h of starvation without food and water, the mice were fed 1 ml of 20% (wt/vol) sucrose containing LB-grown *E. coli* MG1655 strains, as described in the Results. After ingestion of the bacterial suspension, both the food (Harlan Teklad mouse and rat diet; Harlan Teklad, Madison, WI) and streptomycin-containing water were returned to the mice, and 1 g of feces was collected after 5 and 24 h and on odd-numbered days at the times indicated below. Mice were housed individually in cages without bedding and were placed in clean cages daily. Fecal samples (no older than 24 h) were homogenized in 1% Bacto tryptone, diluted in the same medium, and plated on MacConkey agar plates with appropriate antibiotics. Plates contained streptomycin sulfate (100 µg/ml) and nalidixic acid (50 µg/ml), streptomycin sulfate (100 µg/ml) and kanamycin sulfate (40 µg/ml), or streptomycin sulfate (100 µg/ml) and chloramphenicol (30 µg/ml). Antibiotics were purchased from Sigma-Aldrich (St. Louis, MO). All plates were incubated for 18 to 24 h at 37°C prior to

counting. Each colonization experiment was performed at least twice, and essentially identical results were obtained. Pooled data from at least two independent experiments are presented below.

Enumeration of *E. coli* MG1655 (-IS1) and *E. coli* MG1655 (+IS1) Δ flhD in mouse small intestine mucus and cecal mucus. On day 16 after three mice were fed *E. coli* MG1655 Δ edd*, *E. coli* MG1655 (-IS1), and *E. coli* MG1655 (+IS1) Δ flhD, the mice were sacrificed by CO₂ asphyxiation, and their ceca and small intestines, exclusive of the ileum, were removed. Cecal mucus and small intestine mucus from each mouse were scraped into 5 ml of HEPES-Hanks' buffer (pH 7.4) as previously described (6). The numbers of *E. coli* MG1655 (-IS1) and *E. coli* MG1655 (+IS1) Δ flhD cells in each sample were determined by dilution and plating on MacConkey agar plates containing streptomycin sulfate (100 µg/ml) and either nalidixic acid (50 µg/ml) to enumerate *E. coli* MG1655 (-IS1) or chloramphenicol (30 µg/ml) to enumerate *E. coli* MG1655 (+IS1) Δ flhD. The plates were incubated for 18 to 24 h at 37°C prior to counting. Counts were corrected for the total volume of each sample.

Statistics. Where indicated below, log₁₀ means of the numbers of CFU/gram of feces of two strains from six mice were compared by Student's *t* test (*P* values). A *P* value of >0.1 was interpreted as indicating no significant difference, and a *P* value of <0.05 was interpreted as indicating a significant difference.

RESULTS

***E. coli* MG1655 (+IS1) and *E. coli* MG1655 (-IS1).** Two different strains of wild-type *E. coli* MG1655 exist. The first strain, CGSC 7440, which has been sequenced (3), has an IS1 element in the regulatory region of the *flhDC* operon, hyper-expresses the *flhDC* operon, and is hypermotile compared to the second strain, CGSC 6300, which is missing the element (2). Previously, a streptomycin-resistant, nalidixic acid-resistant mutant of CGSC 7440, which is referred to in this paper as *E. coli* MG1655 (+IS1), was used to study colonization of the mouse intestine (17). For the purposes of the present investigation, a strain was constructed that is isogenic to *E. coli* MG1655 (+IS1) except that it is missing the IS1 element in the regulatory region of the *flhDC* operon (see Materials and Methods). This strain is referred to in this paper as *E. coli* MG1655 (-IS1). The *flhDC* locus for each of the strains is illustrated in Fig. 1. As expected, *E. coli* MG1655 (+IS1) is hypermotile compared to *E. coli* MG1655 (-IS1) (Fig. 2).

Kinetics of appearance of nonmotile *E. coli* MG1655 (+IS1) Δ flhDC mutants in the mouse intestine. Previously, we reported that the mouse intestine selected nonmotile mutants of *E. coli* MG1655 (+IS1) with deletions beginning immediately downstream of the IS1 element within the regulatory region of the *flhDC* operon that have improved colonizing ability (17). These mutants grew 10 to 20% faster than their parent in mouse cecal mucus in vitro and 15 to 30% faster on several sugars found in the mouse intestine (17). In the present investigation, experiments were performed to determine the kinetics of appearance of these mutants in vivo.

A total of 6,300 *E. coli* MG1655 (+IS1) colonies, plated directly from 42 overnight LB cultures that had each been started from a separate single colony, were tested for motility (150 colonies per culture). All of the colonies were motile. However, when fecal samples from 42 mice fed *E. coli* MG1655 (+IS1) (10⁵ CFU/mouse) from these LB cultures were examined for motility, by day 3 after feeding 45 to 50% of the colonies were nonmotile and by day 15 after feeding between 80 and 90% were nonmotile (Fig. 3). Between 1 and 15 days after feeding the total numbers of *E. coli* MG1655 (+IS1) in feces remained relatively constant (Fig. 3). Ten nonmotile colonies (three from one mouse on day 3 after feeding and one

FIG. 3. Kinetics of appearance of nonmotile *E. coli* MG1655 (+IS1) in the mouse intestine. Forty-two mice were fed 10^5 CFU of wild-type *E. coli* MG1655 (+IS1). At the indicated times, 50 colonies isolated from the feces of each mouse were tested for motility (□) as described in Materials and Methods; the error bars represent the standard deviations of the means for the percentage of nonmotile *E. coli* MG1655 (+IS1). ■, log₁₀ mean *E. coli* MG1655 (+IS1) CFU per gram of feces for 42 mice (the error bars represent the standard errors of the log₁₀ means).

each from seven individual mice on various days after feeding) were examined for deletions in the *flhDC* operon. DNA sequencing revealed that all 10 nonmotile isolates had deletions in the *flhDC* operon beginning at the same base pair immediately downstream of the *IS1* target sequence and ranging in size from 102 to 6,166 bp. However, despite this strong selection for $\Delta flhDC$ mutants, 10 to 20% of the *E. coli* MG1655 colonies remained motile over a 15-day period (Fig. 3), sug-

gesting that there is an as-yet-undefined intestinal niche in which motility is an advantage.

High osmolarity reduces *E. coli* MG1655 (–IS1) motility but not *E. coli* MG1655 (+IS1) motility. In *E. coli* K-12, expression of the *flhDC* operon lacking the *IS1* element decreases drastically with increasing osmolarity due to the repressing effect of phosphorylated OmpR binding to the *flhDC* promoter (37). Phosphorylated OmpR is generated under high-osmolarity conditions (9). The NaCl concentration in the human intestine has been reported to be 140 mM (1), which is considerably higher than that in most laboratory media. To determine whether the hypermotile phenotype of *E. coli* MG1655 (+IS1) is affected by NaCl concentrations found in the intestine, the motilities of *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression) and *E. coli* MG1655 (–IS1) (normally motile, normal *flhDC* expression) were tested with a wide range of NaCl concentrations. *E. coli* MG1655 (+IS1) was twofold more motile than *E. coli* MG1655 (–IS1) at the lowest NaCl concentration and about fourfold more motile at the higher concentrations (Fig. 4). Furthermore, the motility of *E. coli* MG1655 (+IS1) did not decrease to a significant extent with increasing NaCl concentrations, whereas the motility of *E. coli* MG1655 (–IS1) decreased more than twofold between 100 mM NaCl and 200 mM NaCl such that it was nearly nonmotile. It therefore appears that the presence of the *IS1* element in the regulatory region of the *flhDC* operon not only results in hypermotility but also interferes with the negative regulation of the *flhDC* operon that occurs at higher NaCl concentrations, such as those found in the intestine.

Growth on a mixture of 10 sugars. *E. coli* MG1655 (+IS1) $\Delta flhDC$ mutants (nonmotile, no *flhDC* expression), selected in the intestine from the parent *E. coli* MG1655 (+IS1) (hyper-

FIG. 4. Spread of *E. coli* MG1655 (+IS1) (◆) and *E. coli* MG1655 (–IS1) (■) on motility agar with various concentrations of NaCl. Sets of three colonies of each strain were transferred with toothpicks to motility agar containing the indicated concentrations of NaCl as described in Materials and Methods. Incubation was for 5.5 h at 37°C. The data for each strain are expressed as the percentage of the spread observed in the absence of added NaCl. Each data point was corrected for the diameter of growth of the nonmotile *E. coli* MG1655 (+IS1) $\Delta flhD$ mutant on motility agar (1.6 ± 0.3 mm). The corrected spread for *E. coli* MG1655 (+IS1) without added NaCl was 9.4 ± 2 mm, and that for *E. coli* MG1655 (–IS1) was 4.7 ± 0.3 mm. The error bars represent standard deviations.

FIG. 5. Growth of *E. coli* MG1655 (+IS1) (■), *E. coli* MG1655 (-IS1) (▲), *E. coli* MG1655 (+IS1) Δ flhD (○), and *E. coli* MG1655 (+IS1) Δ motAB Δ fliC (□) on a mixture of 10 sugars. Incubation was at 37°C with aeration. The means and standard deviations of the A_{600} values at the indicated times are presented for three independent cultures of each strain.

motile, hyper-*flhDC* expression), grow 15 to 30% faster than their parent on a number of sugars present in mouse cecal mucus and are better colonizers of the mouse intestine than their parent (17). The same is true of an *E. coli* MG1655 (+IS1) Δ flhD mutant (nonmotile, no *flhDC* expression) constructed in the laboratory that contains a 546-bp deletion beginning immediately downstream of ISI in the regulatory region of *flhD* and continuing into the *flhD* gene (17). Because the promoter is deleted, the entire *flhDC* operon is inactivated, but since the deletion ends in *flhD*, the strain is referred to as *E. coli* MG1655 (+IS1) Δ flhD. To determine how well *E. coli* MG1655 (-IS1) (normally motile, normal *flhDC* expression) grows relative to *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression) and *E. coli* MG1655 (+IS1) Δ flhD (nonmotile, no *flhDC* expression), the strains were grown in M9 minimal medium containing 200 mM NaCl to mimic the salt concentration in the intestine and a mixture of 10 sugars (L-arabinose, L-fucose, D-galactose, D-gluconate, N-acetyl-D-glucosamine, D-glucuronate, maltose, D-mannose, D-melibiose, and D-ribose), each at a concentration of 0.005%. As shown in Fig. 5, *E. coli* MG1655 (+IS1) Δ flhD (nonmotile, no *flhDC* expression) and *E. coli* MG1655 (-IS1) (normally motile, normal *flhDC* expression) grew at the same rate, and both grew faster than *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression). *E. coli* MG1655 (+IS1) Δ flhD (nonmotile, no *flhDC* expression) and *E. coli* MG1655 (-IS1) (normally motile, normal *flhDC* expression) both reached a higher final absorbance than *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression) (Fig. 4). It might be argued that hyperflagellation causes *E. coli* MG1655 (+IS1) to clump more than the other strains, which would lower its absorbance readings; however, when the organisms were viewed microscopically, none of the strains clumped at any time during growth. Therefore, it appeared that either the reduced expression of the *flhDC* operon in the normally motile strain *E. coli* MG1655 (-IS1) compared to the expression in the hypermotile strain *E. coli* MG1655 (+IS1) or the reduced amount of energy required for

flagellum synthesis and rotation in the normally motile strain *E. coli* MG1655 (-IS1) compared to the amount required in the hypermotile strain *E. coli* MG1655 (+IS1) was responsible for the higher growth rate and higher growth yield of strain *E. coli* MG1655 (+IS1). Indeed, the energy required to synthesize flagella and rotate the motor has been estimated to be about 2% of the total energy that is normally consumed during growth (21). To distinguish between the two possibilities, the growth rate on the mixture of 10 sugars of *E. coli* MG1655 (+IS1) Δ motAB Δ fliC (nonmotile, hyper-*flhDC* expression), which is unable to synthesize flagellin or rotate the flagellar motor, was also determined in the experiment depicted in Fig. 5. *E. coli* MG1655 (+IS1) Δ motAB Δ fliC (nonmotile, hyper-*flhDC* expression) grew as rapidly as *E. coli* MG1655 (+IS1) Δ flhD (nonmotile, no *flhDC* expression) and *E. coli* (-IS1) (normally motile, normal *flhDC* expression) and faster and with a higher final yield than *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression). Therefore, the growth data suggest that it is the energy required to hypersynthesize and rotate its flagella rather than hyper-*flhDC* expression that slows the growth of *E. coli* MG1655 (+IS1) compared to the growth of the other three strains in minimal medium containing the 10-sugar mixture.

Mouse-colonizing abilities of *E. coli* MG1655 (+IS1), *E. coli* MG1655 (+IS1) Δ flhD, *E. coli* MG1655 (-IS1), and *E. coli* MG1655 (+IS1) Δ motAB Δ fliC. *E. coli* MG1655 Δ edd is defective in the Entner-Doudoroff pathway and is motile. *E. coli* MG1655 Δ edd* is also defective in the Entner-Doudoroff pathway, was selected by the mouse intestine as being a better colonizer than its *E. coli* MG1655 Δ edd parent, and was found to be nonmotile due to deletion of the entire *flhDC* operon (17). It also grew faster than both its parent and *E. coli* MG1655 (+IS1) on a number of sugars found in the mouse intestine (5, 17). When low numbers (10^5 CFU/mouse) were fed to mice, *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression) was eliminated from the mouse intestine by high numbers (10^{10} CFU/mouse) of *E. coli* MG1655 Δ edd* (17), but low numbers (10^5 CFU/mouse) of *E. coli* MG1655 (+IS1) Δ flhD (nonmotile, no *flhDC* expression) grew to high numbers in mice fed high numbers (10^{10} CFU/mouse) of *E. coli* MG1655 Δ edd* (17). These results showed that *E. coli* MG1655 (+IS1) Δ flhD (nonmotile, no *flhDC* expression) was a better colonizer than *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression) (17). In the present study, using the ability to grow from low numbers to high numbers in the presence of high numbers of *E. coli* MG1655 Δ edd* in the mouse intestine as a measure of colonizing ability, a series of experiments was conducted to determine whether the increased intestine-colonizing ability of *E. coli* MG1655 (+IS1) Δ flhD (nonmotile, no *flhDC* expression) relative to that of *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression) was due solely to the increase in energy available for growth when hypermotility was prevented by the deletion.

Mice were fed high numbers (10^{10} CFU/mouse) of *E. coli* MG1655 Δ edd* along with low numbers (10^5 CFU/mouse) of the strains to be tested. As observed previously (17), *E. coli* MG1655 (+IS1) Δ flhD (nonmotile, no *flhDC* expression) was a better colonizer than *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression) ($P > 0.1$ at 5 h after feeding, but $P < 0.001$ at both 5 and 7 days after feeding); i.e., *E. coli* MG1655

FIG. 6. Colonization of the mouse intestine. (A) Sets of three mice were fed 10^5 CFU of *E. coli* MG1655 (+IS1) (■), 10^5 CFU of MG1655 (+IS1) Δ flhD (▲), and 10^{10} CFU of *E. coli* MG1655 Δ edd* (●). (B) Sets of three mice were fed 10^5 CFU of *E. coli* MG1655 (+IS1) Δ flhD (▲), and 10^{10} CFU of *E. coli* MG1655 Δ edd* (●). (C) Sets of three mice were fed 10^5 CFU of *E. coli* MG1655 (+IS1) (□), 10^5 CFU of MG1655 (+IS1) Δ motAB Δ fliC (○), and 10^{10} CFU of *E. coli* MG1655 (+IS1) Δ edd* (●). (D) Sets of three mice were fed 10^5 CFU of *E. coli* MG1655 (+IS1) Δ motAB Δ fliC (○), and 10^{10} CFU of *E. coli* MG1655 (+IS1) Δ edd* (●). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Error bars representing standard errors of the log_{10} mean CFU per gram of feces for six mice are presented for each time point.

(+IS1) (hypermotile, hyper-*flhDC* expression) was eliminated from the intestine in the presence of high numbers of *E. coli* MG1655 Δ edd*, whereas *E. coli* MG1655 (+IS1) Δ flhD (nonmotile, no *flhDC* expression) grew to high numbers (Fig. 6A). Compared to nonmotility and no *flhDC* expression, normal motility and normal *flhDC* expression did not detract from colonizing ability; that is, *E. coli* MG1655 (–IS1) (normally motile, normal *flhDC* expression) and *E. coli* MG1655 (+IS1) Δ flhD (nonmotile, no *flhDC* expression) both grew from low to high numbers at the same rate in the presence of high numbers of *E. coli* MG1655 Δ edd* (Fig. 6B) ($P > 0.1$ at 5 h and 5 and 7 days after feeding). As suggested by the growth studies (Fig. 5), energy directed to hyper-flagellum synthesis and flagellar motor operation did indeed appear to decrease colonizing

ability; i.e., *E. coli* MG1655 (+IS1) Δ motAB Δ fliC (nonmotile, hyper-*flhDC* expression) was a better colonizer than *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression) ($P > 0.1$ at 5 h after feeding, but $P < 0.02$ at 5 days after feeding and $P < 0.05$ at 7 days after feeding) (Fig. 6C). However, it appears that hyperexpression of *flhDC*, independent of the energy expended for hypermotility, also decreases colonizing ability; that is, *E. coli* MG1655 (+IS1) Δ motAB Δ fliC (nonmotile, hyper-*flhDC* expression) was not nearly as good a colonizer as *E. coli* MG1655 (–IS1) (normally motile, normal *flhDC* expression) ($P > 0.1$ at 5 h after feeding, but $P < 0.001$ at 5 and 7 days after feeding) (Fig. 6D). Since normal motility requires more energy expenditure than nonmotility, these data suggest that *E. coli* MG1655 (+IS1) Δ motAB Δ fliC (nonmotile, hyper-*flhDC* ex-

TABLE 1. Summary of strain genotypes, phenotypes, and colonization abilities

<i>E. coli</i> strain	<i>flhDC</i> expression ^a	Motility ^b	Intestine-colonizing ability ^c
MG1655 (+IS1) Δ <i>flhD</i>	–	–	Best
MG1655 (–IS1)	+	+	Best
MG1655 (+IS1)	++	–	Next best
Δ <i>motAB</i> Δ <i>fliC</i>			
MG1655 (+IS1)	++	++	Worst

^a –, no *flhDC* expression; +, normal *flhDC* expression; ++, hyper-*flhDC* expression.

^b –, nonmotile; +, normally motile; ++, hypermotile.

^c See Fig. 6.

pression) is a worse colonizer than *E. coli* MG1655 (–IS1) (normally motile, normal *flhDC* expression) because of regulatory effects of hyperexpression of *flhDC* unrelated to its effects on hyper-flagellum synthesis and rotation. Table 1 summarizes these results.

Growth of *E. coli* MG1655 in mouse small intestine mucus in vivo does not require motility. Although *E. coli* MG1655 (–IS1) (normally motile, normal *flhDC* expression) and *E. coli* MG1655 (+IS1) Δ *flhD* (nonmotile, no *flhDC* expression) appeared to be equally good colonizers (Fig. 6B), it was still possible that growth in a specific segment of the mouse intestine might require that *E. coli* MG1655 strains be motile. If so, that segment should be available to *E. coli* MG1655 (–IS1) (normally motile, normal *flhDC* expression) but not to *E. coli* MG1655 (+IS1) Δ *flhD* (nonmotile, no *flhDC* expression). *E. coli* MG1655 colonizes the mouse intestine by growing in intestinal mucus (24). Since both *E. coli* MG1655 (–IS1) and *E. coli* MG1655 (+IS1) Δ *flhD* appear in equal numbers in feces, it would be expected that they would appear in equal numbers in cecal mucus. The question was whether *E. coli* MG1655 (–IS1) and *E. coli* MG1655 (+IS1) Δ *flhD* appear in equal numbers in small intestine mucus, which harbors far fewer total *E. coli* MG1655 cells than cecal mucus. Therefore, at day 16 after feeding of high numbers of *E. coli* MG1655 *edd** cells and low numbers of *E. coli* MG1655 (–IS1) and *E. coli* MG1655 (+IS1) Δ *flhD* cells, cecal mucus and small intestine mucus, exclusive of the ileum, which mimics cecal mucus, were isolated from the mice and plated to determine the amounts of *E. coli* MG1655 (–IS1) and *E. coli* MG1655 (+IS1) Δ *flhD*. As expected, the amounts of the two strains in cecal mucus were 20- to 30-fold higher than the amounts in the mucus isolated from the entire small intestine (Table 2); however, *E. coli* MG1655 (–IS1) and *E. coli* MG1655 (+IS1) Δ *flhD* were found in equal numbers in cecal mucus and in equal numbers in small intestine mucus (Table 2). It should be noted that all 50 *E. coli* MG1655 (–IS1) colonies isolated from the cecal mucus of each mouse and tested for motility and all 50 *E. coli* MG1655 (–IS1) colonies isolated from the small intestine mucus of each mouse and tested for motility were motile. These data suggest that although *E. coli* MG1655 (–IS1) (normally motile, normal *flhDC* expression) and *E. coli* MG1655 (+IS1) Δ *flhD* (nonmotile, no *flhDC* expression) grow to higher numbers in cecal mucus, they grow equally well relative to one another both in small intestine mucus and in cecal mucus in vivo. It therefore does not appear that growth in small intestinal mucus in vivo requires motility.

TABLE 2. *E. coli* MG1655 (–IS1) and *E. coli* MG1655 Δ *flhDC* in mouse cecal mucus and small intestine mucus

Location	Log ₁₀ CFU ^a	
	<i>E. coli</i> MG1655 (–IS1)	<i>E. coli</i> MG1655 Δ <i>flhDC</i>
Small intestine mucus	3.93 ± 0.26	3.60 ± 0.59
Cecal mucus	5.24 ± 0.39	5.16 ± 0.24
Feces	6.97 ± 0.27	7.18 ± 0.18

^a The values are log₁₀ means ± standard errors of the means for three mice. Mucus preparations were isolated on day 16 after feeding. The CFU values for the small intestine and cecal mucus are corrected for the entire volume of each mucus preparation. The fecal values are the CFU/gram of feces at 15 days after feeding.

DISCUSSION

A large and growing body of evidence indicates that commensal *E. coli* strains grow in the intestine largely on nutrients acquired from cecal mucus and that growth is inhibited in cecal contents (18, 24, 28, 38, 39, 43). The cecal mucus layer of the mouse intestine appears to turn over about every 2 h (33) and is continuously being degraded by the indigenous microflora. The degraded cecal mucus is shed into the cecal contents and ultimately is compacted in feces. If commensal *E. coli* strains do not attach to underlying intestinal epithelial cells (24, 29) and growth takes place predominantly in the mucus layer, it seems reasonable that to maintain a stable population in the intestine, the bacterial growth rate must keep pace with the turnover rate of the mucus layer. In addition, it is becoming increasingly clear that in the face of competition from a complex microflora, *E. coli* MG1655 simultaneously uses several, presumably limiting, sugars for growth in mouse cecal mucus, including L-fucose, D-gluconate, N-acetylglucosamine, and sialic acid (5). As a consequence, any slight advantage that an *E. coli* MG1655 mutant might have in utilizing these nutrients to attain even a small increase in the growth rate would be magnified into predominance of the mutant over the wild type in the intestine over time.

In a previous study, it was shown that *E. coli* MG1655 (+IS1) Δ *flhDC* mutants were selected by the mouse intestine (17). These mutants were better intestinal colonizers than their parent and grew better in vitro in cecal mucus and in vitro on a variety of sugars normally found in cecal mucus (17). The *E. coli* MG1655 (+IS1) Δ *flhDC* mutants were generated with deletions immediately downstream of the IS1 element that is present in the regulatory region of the *flhDC* promoter of *E. coli* MG1655 (+IS1). The data presented here show that although less than 1 in 6,300 of the cells in LB are *E. coli* MG1655 Δ *flhDC* mutants (i.e., < 0.016%), almost 50% of the colonies present in feces at 3 days after feeding and about 90% of the colonies by day 15 days after feeding are Δ *flhDC* mutants (Fig. 3). There appear to be two components that contribute to this strong selection. First, *E. coli* MG1655 (+IS1) is hypermotile, and the energy involved in making and rotating its flagella appears to decrease both its growth rate and its colonizing ability; i.e., *E. coli* MG1655 (+IS1) Δ *motAB* Δ *fliC* (nonmotile, hyper-*flhDC* expression) grows faster than *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression) on the mixture of 10 sugars in vitro (Fig. 5) and is the better colonizer (Fig. 6C), yet the only difference between the two strains is that

E. coli MG1655 (+IS1) Δ *motAB* Δ *fliC* is unable to make flagella and rotate the flagellar motor. Second, it appears that hyperexpression of the FlhD₄C₂ complex in *E. coli* MG1655 (+IS1) may result in negative regulation of one or more as-yet-unidentified genes that contribute to maximum colonizing ability. This is suggested by the fact that although *E. coli* MG1655 (+IS1) Δ *motAB* Δ *fliC* (nonmotile, hyper-*flhDC* expression), *E. coli* MG1655 (−IS1) (normally motile, normal *flhDC* expression), and *E. coli* MG1655 (+IS1) Δ *flhD* (nonmotile, no *flhDC* expression) grow at the same rate on the 10-sugar mixture in vitro (Fig. 5), *E. coli* MG1655 (−IS1) (normally motile, normal *flhDC* expression) and *E. coli* MG1655 (+IS1) Δ *flhD* (nonmotile, no *flhDC* expression) are better colonizers than *E. coli* MG1655 (+IS1) Δ *motAB* Δ *fliC* (nonmotile, hyper-*flhDC* expression) (Table 1 and Fig. 6).

E. coli MG1655 (−IS1) is as good an intestinal colonizer as *E. coli* MG1655 (+IS1) Δ *flhD*, yet it makes the FlhD₄C₂ complex and is motile; however, it is about fourfold less motile than *E. coli* MG1655 (+IS1) because it presumably makes less FlhD₄C₂ complex. The fact that it makes less FlhD₄C₂ complex may account for its better colonizing ability relative to that of *E. coli* MG1655 (+IS1); i.e., the concentration of the FlhD₄C₂ complex in *E. coli* MG1655 (−IS1) may not be high enough to either repress expression of genes unrelated to motility that are important for maximum colonization or to hyperexpress motility genes. If this is true, then normal motility would not be detrimental to colonization and *E. coli* MG1655 (−IS1) might be motile in the intestine. Alternatively, it is possible that the high osmolarity in the intestine shuts off expression of the *E. coli* MG1655 (−IS1) *flhDC* operon, as it appears to do on motility agar plates containing high concentrations of NaCl (Fig. 4). If this is true, *E. coli* MG1655 (+IS1) Δ *flhD* and *E. coli* MG1655 (−IS1) would be functionally equivalent in the intestine (i.e., no *flhDC* expression and no motility).

At the present time, we do not know the identity of the gene(s) that appears to be repressed by the FlhD₄C₂ regulatory complex in *E. coli* MG1655 (+IS1) in the intestine. However, in preliminary experiments, when *E. coli* MG1655 (+IS1) (hypermotile, hyper-*flhDC* expression) and *E. coli* MG1655 (+IS1) Δ *flhD* (nonmotile, no *flhDC* expression) were grown in cecal mucus in vitro and the gene expression patterns were compared, it was found that several genes encoding catabolic enzymes for sugars not present in the 10-sugar mixture were upregulated at least twofold in *E. coli* MG1655 (+IS1) Δ *flhD*. These genes were *lacZ* (lactose), *garD* (galactarate), *gudX* (glucarate [putative]), and *garR* (glucarate and galactarate). Indeed, it will be of great interest to examine the roles of lactose, glucarate, and galactarate catabolism in colonization of the mouse intestine by *E. coli* MG1655 (−IS1) and *E. coli* MG1655 (+IS1) Δ *flhDC* mutants.

While we have shown that the mouse small intestine does not appear to be a distinct intestinal segment in which motility is required, in mice fed *E. coli* MG1655 (+IS1) 10 to 20% of the cells remained motile between days 9 and 15 after feeding (Fig. 3). It therefore appears that motility may be favored in a small niche that is present in the mouse intestine. One possibility for the site of this niche is the part of the intestinal mucus layer that is closest to the epithelium; i.e., it may be that motility is required for strains of *E. coli* MG1655 to reach and remain at that site. In addition, the mucosal tissues are oxygen

rich, and it is well known that oxygen diffuses into the intestine at appreciable levels (13, 19). It is therefore likely that *E. coli* MG1655 (+IS1) encounters a higher oxygen tension close to the epithelium than at the luminal surface. Since a major source of nutrients in the intestine could be derived from exfoliated lysed epithelial cells, it is also possible that *E. coli* MG1655 (+IS1) encounters a higher concentration of nutrients closer to the epithelium than at the luminal surface. Nutrient stress has been reported to increase the frequency of transposition mediated by insertion elements (42). Therefore, since the environment close to the epithelium may more closely approximate the favorable growth conditions in LB, it may be that *flhDC* deletions do not occur at an appreciable rate close to the surface of the epithelium or that *flhDC* deletion mutants are not successful in that niche because they are not motile.

In contrast to the situation in *E. coli* MG1655, it appears that motility and chemotaxis do play a major role in salmonellae penetrating the mucus layer in the ileum (35, 45). Moreover, it appears that motility and/or chemotaxis is required for *Campylobacter jejuni* colonization of the suckling mouse intestine (40), for *Helicobacter pylori* colonization of both the mouse and gnotobiotic piglet stomachs (10), and for rapid penetration of rabbit ileal mucus by *Vibrio cholerae* (11). However, it is becoming clear that in some instances, motility is important at some but not all stages of the life cycle of a pathogen and that flagella can sometimes be important for adhesion to mucosal surfaces rather than for motility (12). Nevertheless, it should be noted that in the strain collection of the Statens Serum Institut in Copenhagen, Denmark, 370 of 1,000 enteropathogenic *E. coli* strains are nonmotile (37%), 125 of 520 enterotoxigenic *E. coli* strains are nonmotile (24%), 45 of 135 enteroinvasive *E. coli* strains are nonmotile (33%), and 548 of 1,890 enterohemorrhagic strains are nonmotile (29%) (Karen A. Krogfelt, personal communication).

It should also be noted that selection of *E. coli* MG1655 *flhDC* deletion mutants in the mouse intestine is not the only instance in which mutation within the *flhDC* operon has been reported to occur in nature or to be advantageous. For example, *E. coli* O157:H[−] nonmotile strains, found in up to 40% of human hemolytic-uremic syndrome cases in Germany, have recently been shown to contain a 12-bp deletion in *flhC* (26). In addition, an *flhD* mutant of *Salmonella enterica* serovar Typhimurium was more virulent than its parent in C57BL/6J mice and appeared to grow more rapidly than its wild-type parent in the spleen and in mouse macrophages in tissue culture (36). Moreover, deletion of the *Yersinia enterocolitica flhDC* operon has been reported to upregulate the *yop* (*Yersinia* outer protein) regulon (4, 14). Lastly, it appears that a major reason that *Shigella* strains are nonmotile is damage in the *flhDC* operon caused by either insertions or deletions associated with insertion elements that have occurred during niche adaptation (41).

In summary, the data presented here underscore the complexity of the role of the *flhDC* operon and motility in *E. coli* MG1655 colonization of the mouse intestine. It appears that while motility is not necessary for colonization, there is an as-yet-undefined intestinal niche in which 10 to 20% of *E. coli* MG1655 (+IS1) cells reside and in which motility is an advantage. Identifying where that niche is may shed light on why motility is important for its occupation by *E. coli*

MG1655 (+ISI) and whether occupation of the niche might also be important for pathogens. The *E. coli* MG1655 (+ISI) *flhDC* deletion mutants that are selected by the intestine appear to be selected for two reasons. First, genes unrelated to motility that are either directly or indirectly repressed by FlhD₄C₂ but can contribute to maximum colonizing ability are released from repression. Second, energy normally used to hyperexpress and rotate flagella is redirected to growth.

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REFERENCES

- Banwell, J. G., S. L. Gorbach, N. F. Pierce, R. Mitra, and A. Mondal. 1971. Acute undifferentiated human diarrhea in the tropics. II. Alterations in intestinal fluid and electrolyte movements. *J. Clin. Invest.* **50**:890–900.
- Barker, C. S., B. M. Prüß, and P. Matsumura. 2004. Increased motility of *Escherichia coli* by insertion sequence element integration into the regulatory region of the *flhD* operon. *J. Bacteriol.* **186**:7529–7537.
- Blattner, F. R., G. Plunkett III, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* **277**:1453–1474.
- Bleves, S., M. Marie-Noëlle, G. Detry, and G. R. Cornelis. 2002. Up-regulation of the *Yersinia enterocolitica* *yop* regulon by deletion of the flagellum master operon *flhDC*. *J. Bacteriol.* **184**:3214–3223.
- Chang, D. E., D. J. Smalley, D. L. Tucker, M. P. Leatham, W. E. Norris, S. J. Stevenson, A. B. Anderson, J. E. Grissom, D. C. Laux, P. S. Cohen, and T. Conway. 2004. Carbon nutrition of *Escherichia coli* in the mouse intestine. *Proc. Natl. Acad. Sci. USA* **101**:7427–7432.
- Cohen, P. S., and D. C. Laux. 1995. Bacterial adhesion to and penetration of intestinal mucus *in vitro*. *Methods Enzymol.* **253**:309–315.
- Conway, K. A. Krogfelt, and P. S. Cohen. 29 December 2004, posting date. The life of commensal *Escherichia coli* in the mammalian intestine. In R. Curtiss III, et al. (ed.), *EcoSal—Escherichia coli and Salmonella: cellular and molecular biology*. ASM Press, Washington, DC. <http://www.ecosal.org>.
- Datsenko, K. A., and B. L. Wanner. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* **97**:6640–6645.
- Egger, L. A., H. Park, and M. Inouye. 1997. Signal transduction via the histidyl-aspartyl phosphorelay. *Genes Cells* **2**:167–184.
- Foynes, S., N. Dorrell, S. J. Ward, R. A. Stabler, A. A. McColl, A. N. Rycroft, and B. W. Wren. 2000. *Helicobacter pylori* possesses two CheY response regulators and a histidine kinase sensor, CheA, which are essential for chemotaxis and colonization of the gastric mucosa. *Infect. Immun.* **68**:2016–2023.
- Freter, R., P. C. O'Brien, and M. S. Macsai. 1981. Role of chemotaxis in the association of motile bacteria with intestinal mucosa: *in vivo* studies. *Infect. Immun.* **34**:234–240.
- Girón, J. A. 2005. Role of flagella in mucosal colonization, p. 213–235. In J. P. Nataro, P. S. Cohen, H. L. T. Mobley, and J. N. Weiser (ed.), *Colonization of mucosal surfaces*. American Society for Microbiology Press, Washington, DC.
- He, G., R. A. Shankar, M. Chzhan, A. Samouilov, P. Kuppusamy, and J. L. Zweier. 1999. Noninvasive measurement of anatomic structure and intraluminal oxygenation in the gastrointestinal tract of living mice with spatial and spectral EPR imaging. *Proc. Natl. Acad. Sci. USA* **96**:4586–4591.
- Horne, S. M., and B. M. Prüß. 2006. Global gene regulation in *Yersinia enterocolitica*: effect of FlhA on the expression levels of flagellar and plasmid-encoded virulence genes. *Arch. Microbiol.* **185**:115–126.
- Hoskins, L. 1984. Mucin degradation by enteric bacteria: ecological aspects and implications for bacterial attachment to gut mucosa, p. 51–65. In E. C. Boedeker (ed.), *Attachment of organisms to the gut mucosa*, vol. II. CRC Press, Inc., Boca Raton, FL.
- Laux, D. C., P. S. Cohen, and T. Conway. 2005. Role of the mucus layer in bacterial colonization of the intestine, p. 199–212. In J. P. Nataro, P. S. Cohen, H. L. T. Mobley, and J. N. Weiser (ed.), *Colonization of mucosal surfaces*. American Society for Microbiology Press, Washington, DC.
- Leatham, M. P., S. J. Stevenson, E. J. Gauger, K. A. Krogfelt, J. J. Lins, T. L. Haddock, S. M. Autieri, T. Conway, and P. S. Cohen. 2005. The mouse intestine selects nonmotile *flhDC* mutants of *Escherichia coli* MG1655 with increased colonizing ability and better utilization of carbon sources. *Infect. Immun.* **73**:8039–8049.
- Licht, T. R., T. Tøker-Nielsen, K. Holmstrøm, K. A. Krogfelt, and S. Molin. 1999. Inhibition of *Escherichia coli* precursor 16S rRNA processing by mouse intestinal contents. *Environ. Microbiol.* **1**:23–32.
- Liévin-Le Moal, V., and A. L. Servin. 2006. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. *Clin. Microbiol. Rev.* **19**:315–337.
- Liu, X., and P. Matsumura. 1994. The FlhD/FlhC complex, a transcriptional activator of the *Escherichia coli* flagellar class II operons. *J. Bacteriol.* **176**:7345–7351.
- Macnab, R. M. 1996. Flagella and motility, p. 123–145. In F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli and Salmonella: cellular and molecular biology*, 2nd ed., vol. 1. American Society for Microbiology Press, Washington, DC.
- Miller, C. P., and M. Bohnhoff. 1963. Changes in the mouse's enteric microflora associated with enhanced susceptibility to *Salmonella* infection following streptomycin-treatment. *J. Infect. Dis.* **113**:59–66.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Miranda, R. L., T. Conway, M. P. Leatham, D. E. Chang, W. E. Norris, J. H. Allen, S. J. Stevenson, D. C. Laux, and P. S. Cohen. 2004. Glycolytic and gluconeogenic growth of *Escherichia coli* O157:H7 (EDL933) and *E. coli* K-12 (MG1655) in the mouse intestine. *Infect. Immun.* **72**:1666–1676.
- Møller, A. K., M. P. Leatham, T. Conway, P. J. M. Nuijten, L. A. M. de Haan, K. A. Krogfelt, and P. S. Cohen. 2003. An *Escherichia coli* MG1655 lipopolysaccharide deep-rough core mutant grows and survives in mouse cecal mucus but fails to colonize the mouse large intestine. *Infect. Immun.* **71**:2142–2152.
- Monday, S. R., S. A. Minnich, and P. C. Feng. 2004. A 12-base-pair deletion in the flagellar master control gene *flhC* causes nonmotility of the pathogenic German sorbitol-fermenting *Escherichia coli* O157:H[−] strains. *J. Bacteriol.* **186**:2319–2327.
- Neutra, M. R. 1984. The mechanism of intestinal mucous secretion, p. 33–41. In E. C. Boedeker (ed.), *Attachment of organisms to the gut mucosa*, vol. II. CRC Press, Inc., Boca Raton, FL.
- Newman, J. V., R. Kolter, D. C. Laux, and P. S. Cohen. 1994. The role of *leuX* in *Escherichia coli* colonization of the streptomycin-treated mouse large intestine. *Microb. Pathog.* **17**:301–311.
- Poulsen, L. K., F. Lan, C. S. Kristensen, P. Hobolth, S. Molin, and K. A. Krogfelt. 1994. Spatial distribution of *Escherichia coli* in the mouse large intestine from rRNA *in situ* hybridization. *Infect. Immun.* **62**:5191–5194.
- Prüß, B. M. 2000. FlhD, a transcriptional regulator in bacteria. *Recent Res. Dev. Microbiol.* **4**:31–42.
- Prüß, B. M., J. W. Campbell, T. K. Van Dyk, C. Zhu, Y. Kogan, and P. Matsumura. 2003. FlhD/FlhC is a regulator of anaerobic respiration and the Entner-Doudoroff pathway through induction of the methyl-accepting chemotaxis protein Aer. *J. Bacteriol.* **185**:534–543.
- Prüß, B. M., X. Liu, W. Hendrickson, and P. Matsumura. 2001. FlhD/FlhC-regulated promoters analyzed by gene array and lacZ gene fusions. *FEMS Microbiol. Lett.* **197**:91–97.
- Rang, C. U., T. R. Licht, T. Midtvedt, P. L. Conway, L. Chao, K. A. Krogfelt, P. S. Cohen, and S. Molin. 1999. Estimation of growth rates of *Escherichia coli* BJ4 in streptomycin-treated and previously germfree mice by *in situ* rRNA hybridization. *Clin. Diagn. Lab. Immunol.* **6**:434–436.
- Revel, H. R. 1967. Restriction and nonglucosylated T-even bacteriophage: properties of permissive mutants of *Escherichia coli* B and K12. *Virology* **31**:688–701.
- Robertson, J. M., G. Grant, E. Allen-Bercoe, M. J. Woodward, A. Pusztai, and H. J. Flint. 2000. Adhesion of *Salmonella enterica* var. Enteritidis strains lacking fimbriae and flagella to rat ileal explants cultured at the air interface or submerged in tissue culture medium. *J. Med. Microbiol.* **49**:691–696.
- Schmitt, C. K., J. S. Ikeda, S. C. Darnell, P. R. Watson, J. Bispham, T. S. Wallis, D. L. Weinstein, E. S. Metcalf, and A. D. O'Brien. 2001. Absence of all components of the flagellar export and synthesis machinery differentially alters virulence of *Salmonella enterica* serovar Typhimurium in models of typhoid fever, survival in macrophages, tissue culture invasiveness, and calf enterocolitis. *Infect. Immun.* **69**:5619–5625.
- Shin, S., and C. Park. 1995. Modulation of flagellar expression in *Escherichia coli* by acetyl phosphate and the osmoregulator OmpR. *J. Bacteriol.* **177**:4696–4702.
- Sweeney, N. J., P. Klemm, B. A. McCormick, E. Møller-Nielsen, M. Utley, M. A. Schembri, D. C. Laux, and P. S. Cohen. 1996. The *Escherichia coli* K-12 *gntP* gene allows *E. coli* F-18 to occupy a distinct nutritional niche in the streptomycin-treated mouse large intestine. *Infect. Immun.* **64**:3497–3503.
- Sweeney, N. J., D. C. Laux, and P. S. Cohen. 1996. *Escherichia coli* F-18 and K-12 *eda* mutants do not colonize the streptomycin-treated mouse large intestine. *Infect. Immun.* **64**:3504–3511.

40. Takata, T., S. Fujimoto, and K. Amako. 1992. Isolation of nonchemotactic mutants of *Campylobacter jejuni* and their colonization of the mouse intestinal tract. *Infect. Immun.* **60**:3596–3600.
41. Tominaga, A., R. Lan, and P. R. Reeves. 2005. Evolutionary changes of the *flhDC* flagellar master operon in *Shigella* strains. *J. Bacteriol.* **187**:4295–4302.
42. Twiss, E., A. M. Coros, N. P. Tavakoli, and K. M. Derbyshire. 2005. Transposition is modulated by a diverse set of host factors in *Escherichia coli* and is stimulated by nutritional stress. *Mol. Microbiol.* **57**:1593–1607.
43. Wadolkowski, E. A., D. C. Laux, and P. S. Cohen. 1988. Colonization of the streptomycin-treated mouse large intestine by a human fecal *Escherichia coli* strain: role of growth in mucus. *Infect. Immun.* **56**:1030–1035.
44. Wang, S., R. T. Fleming, E. M. Westbrook, P. Matsumura, and D. B. McKay. 2006. Structure of the *Escherichia coli* FlhDC complex, a prokaryotic heteromeric regulator of transcription. *J. Mol. Biol.* **355**:798–808.
45. Worton, K. J., D. C. Candy, T. S. Wallis, G. J. Clarke, M. P. Osborne, S. J. Haddon, and J. Stephen. 1989. Studies on early association of *Salmonella typhimurium* with intestinal mucosa in vivo and in vitro: relationship to virulence. *J. Med. Microbiol.* **29**:283–294.

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