2012

# The Streptomycin-Treated Mouse Intestine Selects *Escherichia coli envZ* Missense Mutants That Interact with Dense and Diverse Intestinal Microbiota

Mary P. Leatham-Jensen
*University of Rhode Island*

Jakob Frimodt-Møller

***See next page for additional authors***



Citation/Publisher Attribution

Leatham-Jensen, M. P., Frimodt-Møller, J., Adediran, J., Mokszycki, M. E., Banner, M. E., Caughron, J. E.,...Cohen, P. S. (2012). The Streptomycin-Treated Mouse Intestine Selects *Escherichia coli envZ* Missense Mutants That Interact with Dense and Diverse Intestinal Microbiota. *Infection and Immunity,80*(5), 1716-1727. doi: 10.1128/IAI.06193-11
Available at: http://dx.doi.org/10.1128/IAI.06193-11

IAI Journals.ASM.org

# The Streptomycin-Treated Mouse Intestine Selects *Escherichia coli envZ* Missense Mutants That Interact with Dense and Diverse Intestinal Microbiota

**Mary P. Leatham-Jensen,[a] Jakob Frimodt-Møller,[b] Jimmy Adediran,[a] Matthew E. Mokszycki,[a] Megan E. Banner,[a] Joyce E. Caughron,[c] Karen A. Krogfelt,[b] Tyrrell Conway,[c] and Paul S. Cohen[a]**

Department of Cell and Molecular Biology, University of Rhode Island, Kingston, Rhode Island, USA[a]; Department of Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen S, Denmark[b]; and Department of Botany and Microbiology, University of Oklahoma, Norman, Okloahoma, USA[c]

**Previously, we reported that the streptomycin-treated mouse intestine selected nonmotile *Escherichia coli* MG1655 *flhDC* deletion mutants of *E. coli* MG1655 with improved colonizing ability that grow 15% faster *in vitro* in mouse cecal mucus and 15 to 30% faster on sugars present in mucus (M. P. Leatham et al., Infect. Immun. 73:8039–8049, 2005). Here, we report that the 10 to 20% remaining motile *E. coli* MG1655 are *envZ* missense mutants that are also better colonizers of the mouse intestine than *E. coli* MG1655. One of the *flhDC* mutants, *E. coli* MG1655 Δ*flhD*, and one of the *envZ* missense mutants, *E. coli* MG1655 mot-1, were studied further. *E. coli* MG1655 mot-1 is more resistant to bile salts and colicin V than *E. coli* MG1655 Δ*flhD* and grows ca. 15% slower *in vitro* in mouse cecal mucus and on several sugars present in mucus compared to *E. coli* MG1655 Δ*flhD* but grows 30% faster on galactose. Moreover, *E. coli* MG1655 mot-1 and *E. coli* MG1655 Δ*flhD* appear to colonize equally well in one intestinal niche, but *E. coli* MG1655 mot-1 appears to use galactose to colonize a second, smaller intestinal niche either not colonized or colonized poorly by *E. coli* MG1655 Δ*flhD*. Evidence is also presented that *E. coli* MG1655 is a minority member of mixed bacterial biofilms in the mucus layer of the streptomycin-treated mouse intestine. We offer a hypothesis, which we call the "Restaurant" hypothesis, that explains how nutrient acquisition in different biofilms comprised of different anaerobes can account for our results.**

When a bacterial species indefinitely persists in stable numbers in the intestine of an animal, without repeated introduction, the animal is, by definition, colonized by the bacterium. Commensal *Escherichia coli* strains colonize the human intestine in the presence of a dense and diverse intestinal microbiota comprised of at least 500 cultivable species and $10^{13}$ to $10^{14}$ total bacteria (15). Unfortunately, *E. coli* colonization cannot be studied experimentally in conventional animals due to colonization resistance, which results when all niches are filled by the microbiota (10). Such experiments require an animal model with open niches for *E. coli* to colonize in relatively high numbers but must also have a dense and diverse anaerobic community that matches as closely as possible the native microbiota of the conventional animal. The streptomycin-treated mouse model is used routinely for this purpose (11, 34).

In mammals, a large fraction of the intestinal microbiota belongs to either of two phyla, the *Firmicutes* and the *Bacteroidetes* (16, 37, 58). Extensive microbiological characterization of the microbial community in the streptomycin-treated mouse intestine, published in the classic paper by Hentges et al. (28), demonstrated that streptomycin treatment (5 mg/ml in drinking water) selectively removes the facultative anaerobes, with much less impact on the anaerobes. Streptomycin treatment does not alter the total numbers of *Bacteroides* (*Bacteroidetes*), *Eubacterium* (*Firmicutes*), *Bifidobacterium* (*Actinobacteria*), and *Clostridium* (*Firmicutes*), although there is a large decrease in *Lactobacillus* (*Firmicutes*) and *Actinomyces* (*Actinobacteria*) with a corresponding increase in *Fusobacterium* (*Fusobacteria*) and an apparent 50% decline in anaerobic species, as indicated by anaerobic colony morphologies (28). In general, more recent studies using 16S rRNA gene sequencing confirmed these results (23, 58, 68). It is now clear that any strategy to overcome colonization resistance will impact community structure (12, 57). While the streptomycin-treated mouse model is not perfect, it allows competition of a variety of *E. coli* strains against a dense and diverse anaerobe population over extended time periods.

Previously, we found that when *E. coli* MG1655 was fed to streptomycin-treated mice, 80 to 90% of the population lacked flagella and became nonmotile within 7 to 9 days postfeeding (24, 36). We showed that these nonmotile better colonizing mutants have deletions of various sizes beginning downstream of an IS*1* element in the *flhDC* regulatory region and extending into or beyond the *flhDC* structural genes (24, 36). FlhD and FlhC form the $FlhD_4C_2$ complex (5, 40, 66), which activates transcription of class II flagellar genes that encode components of the flagellar basal body and export machinery (51). The IS*1* element upstream of the *E. coli* MG1655 *flhDC* promoter increases expression of the *flhDC* operon and makes *E. coli* MG1655 hypermotile (4, 24).

The *flhDC* deletion mutants selected by the streptomycin-treated mouse intestine were found to grow faster than the parent strain *in vitro* in mouse cecal mucus and on sugars normally found in the mucus layer of the intestine (24, 36), which presumably

Received 15 November 2011 Returned for modification 16 December 2011
Accepted 16 February 2012
Published ahead of print 5 March 2012
Editor: B. A. McCormick
Address correspondence to Paul S. Cohen, pco1697u@mail.uri.edu.

doi:10.1128/IAI.06193-11

**TABLE 1** Bacterial strains

| *E. coli* strain | Genotype and/or phenotype[a] | Abbreviated form | Source or reference |
|---|---|---|---|
| MG1655 Str$^r$ | Spontaneous streptomycin resistant mutant of MG1655 | MG1655 | 45 |
| MG1655 Str$^r$ Δ*flhD*::*cat* | 546-bp deletion beginning immediately downstream of IS*1* in the regulatory region of *flhD* and ending in *flhD*, streptomycin and chloramphenicol resistant | MG1655 Δ*flhD* | 36 |
| MG1655-IS*1* Str$^r$ Nal$^r$ | Spontaneous nalidixic acid-resistant mutant of MG1655 -IS*1* Str$^r$, no IS*1* element in the *flhDC* promoter | MG1655-IS*1* | 24 |
| MG1655 Str$^r$ mot-1 | P41L *envZ* missense mutant of MG1655 Str$^r$ | MG1655 mot-1 | This study |
| MG1655 Str$^r$ mot-2 | P148S *envZ* missense mutant of MG1655 Str$^r$ | MG1655 mot-2 | This study |
| MG1655 Str$^r$ mot-3 | V33E *envZ* missense mutant of MG1655 Str$^r$ | MG1655 mot-3 | This study |
| MG1655 Str$^r$ Nal$^r$ mot-1 | Spontaneous nalidixic acid-resistant mutant of MG1655 Str$^r$ mot-1 | MG1655 mot-1 | This study |
| MG1655 Str$^r$ Nal$^r$ mot-1 restored | MG1655 Str$^r$ mot-1 restored to wild type with respect to EnvZ | MG1655 mot-1 restored | This study |
| MG1655 Str$^r$ Nal$^r$ mot-1 Δ*galK*::*cat* | 912-bp deletion in galactokinase gene replaced by a chloramphenicol resistance cassette | MG1655 mot-1 Δ*galK* | This study |
| MG1655 Str$^r$ Nal$^r$ mot-1 Δ*galK*::*cat* restored | MG1655 Str$^r$ Nal$^r$ mot-1 Δ*galK*::*cat* restored to wild type with respect to *galK* | MG1655 mot-1 Δ*galK* restored to wild type with respect to *galK* | This study |
| F-18 Str$^r$ Rif$^r$ | Spontaneous streptomycin- and nalidixic acid-resistant mutant of F-18 | F-18 | 47 |
| BW37751(pKD267)::Kn$^r$ | The plasmid in this strain contains *parE* under the control of the rhamnose promoter and the kanamycin resistance gene | pKD267 | Barry Wanner |

[a] Str$^r$, streptomycin resistance; Kn$^r$, kanamycin resistance; Nal$^r$, nalidixic acid resistance; Rif$^r$, rifampin resistance.

explains why they were selected. Additional studies suggested the *E. coli* MG1655 *flhDC* operon deletion mutants utilize sugars better than their parent because a number of metabolic genes are repressed by the $FlhD_4C_2$ regulatory complex, including *gltA* (citrate synthase), *sdhCDAB* (succinate dehydrogenase), *mdh* (malate dehydrogenase), *mglBAC* (galactose transport), and a large number of sugar catabolism operons (20, 52, 53). Also, increased energy is available for other cellular processes in the absence of hyper-flagellum synthesis and rotation (24).

Despite the strong selection for nonmotile *E. coli* MG1655 *flhDC* deletion mutants by the streptomycin-treated mouse intestine, 10 to 20% of the *E. coli* MG1655 remained motile over a 15 day period (24), suggesting either that they too were better colonizing mutants or that an as-yet-undefined intestinal niche exists in which motility is an advantage. Here, we report that the 10 to 20% of *E. coli* MG1655 that remain motile in the streptomycin-treated mouse intestine are *envZ* missense mutants that display increased colonizing ability.

## MATERIALS AND METHODS

**Bacterial strains.** Bacterial strains used in the present study are listed in Table 1. The original *E. coli* strain K-12 was obtained from a stool sample from a convalescing diphtheria patient in Palo Alto, CA, in 1922 (2). The sequenced *E. coli* MG1655 strain (CGSC 7740) was derived from the original K-12 strain, having only been cured of the temperate bacteriophage lambda and the F plasmid by means of UV light and acridine orange treatment (2). It has an IS*1* element in the *flhDC* promoter (6). The *E. coli* MG1655-IS*1* strain used in the present investigation is isogenic to the MG1655 strain but lacks the IS*1* element in the *flhDC* promoter (24). The allelic-exchange method described by Datsenko and Warner (13) was used to construct *E. coli* MG1655 Δ*galK*, which contains a 912-bp deletion, replaced by a chloramphenicol cassette beginning 153 bp downstream of the ATG start codon and ending 78 bp upstream of the TGA stop codon. As expected, the *E. coli* MG1655 mot-1 Δ*galK* strain failed to grow in M9 minimal medium containing 0.4% (wt/vol) galactose as the sole carbon and energy source. *E. coli* MG1655 mot-1 Δ*galK* was restored to Gal$^+$ by allelic replacement (13), selecting for restored growth on M9 minimal galactose agar plates. The restored Gal$^+$ *E. coli* MG1655 mot-1 grew at the same rate as *E. coli* MG1655 mot-1 in M9 galactose minimal medium. The mutated *envZ* gene in *E. coli* MG1655 mot-1 was replaced with the wild-type *envZ* gene using an unpublished allelic replacement strategy developed by Barry Wanner and Kiryl Datsenko at Purdue University (unpublished data). The first step replaced the *E. coli* MG1655 mot-1 *envZ/ompR* locus with a cassette encoding kanamycin resistance (*kan*) and *parE* under the control of the rhamnose promoter (*kan-rha$_P$-parE*). The *parE* gene encodes a DNA gyrase inhibitor, i.e., the toxin of a toxin anti/toxin pair that kills the cell when produced in the absence of its cognate antitoxin (31). The second step replaced the *kan-rha$_P$-parE* cassette with the wild-type *envZ* allele by selecting for growth on M9 minimal agar plates containing 1.0% (wt/vol) rhamnose (which would otherwise kill when rhamnose induces ParE). The double recombination event leaves no scars on the genome. All constructions were confirmed by sequencing.

**Media and growth conditions.** LB broth Lennox (Difco Laboratories, Detroit, MI), LB agar Lennox (Difco), and MacConkey agar (Difco) were used for routine cultivation. SOC medium was prepared as described by Datsenko and Wanner (13). For testing carbon and energy source utilization, M9 minimal medium (44) was modified by addition of 120 mM NaCl to more closely approximate the sodium chloride concentration in the intestine (3). Growth also was tested in cecal mucus (2.5 mg of protein/ml) in HEPES-Hanks buffer (pH 7.0), which contains 137 mM NaCl, the sodium chloride concentration in jejunal and ileal intestinal fluid (3). Cultures were prepared, and growth was monitored as described previously (36).

**Isolation of cecal mucus.** Mouse cecal mucus was isolated as previously described (9). Briefly, mice (5 to 8 weeks old) were fed Teklad mouse and rat diet (Harlan Laboratories, Madison, WI) for 5 days after being received. The drinking water was then replaced with sterile distilled water containing streptomycin sulfate (5 g/liter). After 24 h, the mice were sacrificed by $CO_2$ asphyxiation, and their ceca were removed. The cecal contents were washed out with sterile distilled water, and the cecal mucus was scraped into HEPES-Hanks buffer (pH 7.0), centrifuged, and sterilized by UV irradiation as described previously (9). All animal protocols were

approved by the University Committee on Use and Care of Animals at the University of Rhode Island.

**Motility.** The motility of *E. coli* MG1655 strains was assayed by toothpicking colonies from LB agar Lennox onto Luria motility agar (3.5 g/liter) containing 200 mM NaCl (3). The plates were incubated for 6 h at 37°C, after which spreading was measured from the edge of each colony. For testing the motility of fecal isolates, 50 colonies from fecal platings of each mouse were toothpicked to motility agar made from LB broth Lennox containing 85.5 mM NaCl. The plates were incubated overnight at room temperature. For testing motility of bacteria from intestinal segments, the ileum, cecum, and colon were removed from each mouse and washed extensively with HEPES-Hanks buffer (pH 7.0), and the mucus was scraped into 5 ml of HEPES-Hanks buffer (pH 7.0), as described previously (9). Samples were homogenized by vortexing, plated on MacConkey agar with appropriate antibiotics, and toothpicked onto motility agar, as described above for fecal isolates.

**Growth in the presence of 5% bile salts.** Strains to be tested for bile sensitivity were grown overnight in LB broth Lennox, diluted to an $A_{600}$ of ~0.1 into fresh LB broth Lennox containing 5% (wt/vol) Bacto-Bile Salts No. 3 (Difco Laboratories), and incubated at 37°C with shaking in 125-ml tissue culture bottles.

**Mouse colonization experiments.** The specifics of the streptomycin-treated mouse model used to compare the large intestine colonizing abilities of *E. coli* strains in mice have been described previously (42, 47, 59, 60, 65). Briefly, sets of 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 resident facultative bacteria (43). After 18 h of starvation for food and water, the mice were fed 1 ml of 20% (wt/vol) sucrose containing $10^5$ CFU or $10^{10}$ CFU of LB broth Lennox-grown *E. coli* strains, as described in Results. After ingesting the bacterial suspension, both the food (Teklad mouse and rat diet; Harlan Laboratories) and streptomycin-water were returned to the mice, and 1 g of feces was collected after 5 h, 24 h, and on odd-numbered days thereafter at the indicated times. Mice were housed individually in cages without bedding and were placed in clean cages at 24-h intervals. Individual fecal pellets were therefore no older than 24 h. Each fecal sample (1 g) was homogenized in 10 ml of 1% Bacto tryptone (Difco), diluted in the same medium, and plated on MacConkey agar plates with appropriate antibiotics. When appropriate, 1 ml of a fecal homogenate (sampled after the feces had settled) was centrifuged at 12,000 × *g*, resuspended in 100 μl of 1% Bacto tryptone, and plated on a MacConkey agar plate with the appropriate antibiotics. This procedure increases the sensitivity of the assay from $10^2$ CFU/gram of feces to 10 CFU/per g of feces. To distinguish the various *E. coli* strains in feces, dilutions were plated on lactose MacConkey agar containing either streptomycin sulfate (100 μg/ml), streptomycin sulfate (100 μg/ml) and nalidixic acid (50 μg/ml), or streptomycin sulfate (100 μg/ml) and chloramphenicol (30 μg/ml). Streptomycin sulfate, chloramphenicol, and nalidixic acid were purchased from Sigma-Aldrich (St. Louis, MO). All plates were incubated for 18 to 24 h at 37°C prior to counting. When necessary to distinguish strains, 100 colonies from plates containing streptomycin were toothpicked onto MacConkey agar plates containing streptomycin and nalidixic acid or onto MacConkey agar plates containing streptomycin and chloramphenicol. Each colonization experiment was performed at least twice, with essentially identical results. Pooled data from at least two independent experiments (a total of six mice) are presented in the figures.

**Sequencing.** DNA sequencing was done at the URI Genomics and Sequencing Center, University of Rhode Island, Kingston, using an Applied Biosystems 3130xl genetic analyzer (Applied Biosystems, Foster City CA). A BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) was used in the sequencing reactions. The primers used to amplify the PCR product (2,241 bp) for sequencing the *ompB* locus included the forward primer (upstream of *ompR*) 5′-TAGCTGGTGACGAACGTGAG-3′ and the reverse primer (downstream of *envZ*) 5′-GTAGCGGCTGGTCCGAA-3′. The same primers, as well as 5′-ATCCTGCTCGCTCATCATC-3′, which begins 565 bp from the 3′ end of *envZ*, were used in the sequencing reactions. The primers used for both amplifying the *galK* deletion for allelic exchange and for sequencing to confirm its presence in *E. coli* MG1655 mot-1 Δ*galK* were the forward primer (43 bp upstream of the *galK* coding sequence) 5′-AACAGGCAGCAGAGCGTTTGC-3′ and the reverse primer (53 bp downstream of the *galK* coding sequence) 5′-AGTCCATCAGCGTGACTACCATC-3′. The primers used for the replacement of the Δ*galK* mutation with wild type were the forward primer (44 bp upstream of the *galK* start codon) 5′-AACAGGCAGCAGAGCGTTTGC-3′ and the reverse primer (68 bp downstream of the *galK* stop codon) 5′-AGTCCATCAGCGTGACTACCATC-3′. The primers used for confirming the restoration of the wild-type *galK* gene sequence are the same as those used for restoring the wild-type sequence.

**Colicin V sensitivity.** *E. coli* F-18 produces colicin V (42). *E. coli* MG1655 strains to be tested for sensitivity to colicin V were grown overnight in LB broth Lennox at 37°C with shaking in 125-ml tissue culture bottles. The next day, $10^6$ CFU of each strain to be tested was added to 3 ml of Luria broth Lennox containing 7 g Difco agar (soft agar)/liter, which was then poured onto a 20-ml Luria broth Lennox agar plate. After the soft agar on each plate solidified, *E. coli* F-18 colonies were toothpicked onto each plate and incubated for 18 h at 37°C, and zones of growth inhibition were measured.

**Fluorescence *in situ* hybridization.** The preparation of mouse cecal histological sections, the oligonucleotide probes, hybridization, and confocal microscopy were all as described previously (46).

**Statistics.** Means derived from the indicated number of samples were compared by using the Student *t* test (*P* values). A *P* value of >0.05 was interpreted as indicating no significant difference, and a *P* value of <0.05 was interpreted as indicating a significant difference.

## RESULTS

**Isolation of *E. coli* MG1655 mot-1, mot-2, and mot-3 from the mouse intestine.** Previously, we showed that despite the strong selection for nonmotile *E. coli* MG1655 *flhDC* deletion mutants by the streptomycin-treated mouse intestine, 10 to 20% of the *E. coli* MG1655 cells remained motile over a 15-day period (36). To determine the nature of the population that remained motile, three mice were fed $10^{10}$ CFU of *E. coli* MG1655 and $10^5$ CFU of *E. coli* MG1655 Δ*flhD*. At 5 h and day 1 postfeeding, the level of *E. coli* MG1655 Δ*flhD* in feces was ~5 orders of magnitude lower than *E. coli* MG1655, but by day 7 *E. coli* MG1655 Δ*flhD* had grown to a population of only 1.5 orders of magnitude lower than *E. coli* MG1655 and remained at that level thereafter for the 15 days of the experiment (Fig. 1A). As a control, mice were fed $10^{10}$ CFU of *E. coli* MG1655 Δ*flhD* and $10^5$ CFU of *E. coli* MG1655. As expected, the better colonizing *E. coli* MG1655 Δ*flhD* completely eliminated *E. coli* MG1655 within 3 days postfeeding (Fig. 1B). At day 15, the mice fed high numbers of *E. coli* MG1655 and low numbers of *E. coli* MG1655 Δ*flhD* (Fig. 1A) were sacrificed, and the numbers of *E. coli* MG1655 and *E. coli* MG1655 Δ*flhD* in ileal mucus, cecal mucus, and colonic mucus were determined. In each mucus preparation, the numbers of *E. coli* MG1655 Δ*flhD* were 1.5 to 2 orders of magnitude lower than *E. coli* MG1655, reflecting the results observed in feces (Table 2). Although less than 1 in 6,300 CFU of the *E. coli* MG1655 fed to the mice were nonmotile (24) and less than 1 in 100 CFU in feces were nonmotile at both 5 h and 1 day postfeeding, by day 3 postfeeding 17% ± 7.9% (mean ± the SEM) in feces were nonmotile, by day 7 postfeeding 65% ± 15% in feces were nonmotile, by day 13 postfeeding 75% ± 18% in feces were nonmotile, and on day 15 postfeeding 76% ± 13% in feces were nonmotile. Therefore, despite competition from the better-colonizing *E. coli* MG1655 Δ*flhD* and the

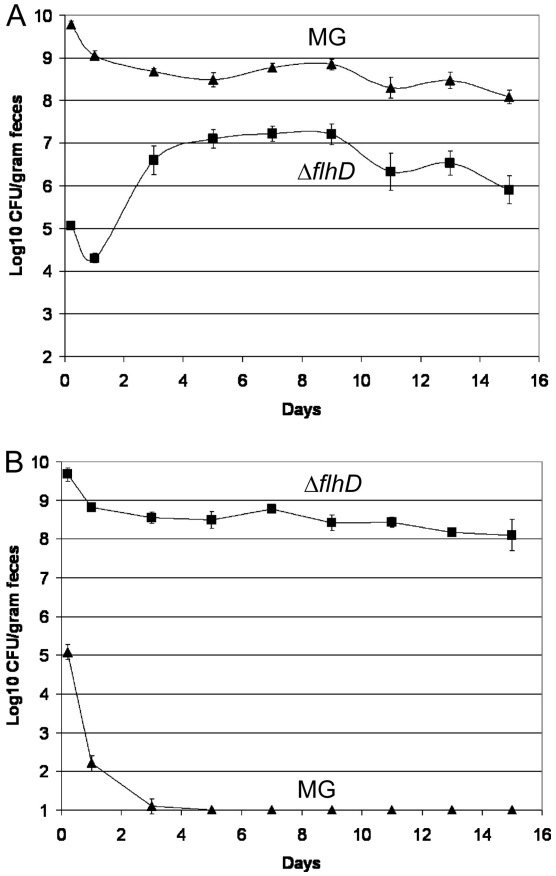


**FIG 1** Colonization of the mouse intestine by *E. coli* MG1655 and *E. coli* MG1655 Δ*flhD*. Three mice were fed $10^{10}$ CFU of *E. coli* MG1655 Str$^r$ (▲) and $10^5$ CFU of MG1655 Str$^r$ Δ*flhD*::*cat* (■) (A), and three mice were fed $10^{10}$ CFU of MG1655 Str$^r$ Δ*flhD*::*cat* (■) and $10^5$ CFU of MG1655 Str$^r$ Nal$^r$ (▲) (B). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars representing standard errors of the $\log_{10}$ means of CFU per g of feces for three mice are presented for each time point.

selection by the intestine of nonmotile *E. coli* MG1655, ca. 25% of the *E. coli* MG1655 remained motile within each section of the intestine. On day 13 postfeeding, 1 motile *E. coli* MG1655 colony was selected from the feces of each mouse for further study. These

**TABLE 2** *E. coli* MG1655 and *E. coli* MG1655 Δ*flhD* and percent nonmotile *E. coli* MG1655 in mouse ileal, cecal, and colonic mucosa at 15 days postfeeding

| Location | Mean $\log_{10}$ CFU ± SEM[a] | | |
|---|---|---|---|
| | MG1655 | MG1655 Δ*flhD* | % Nonmotile MG1655[b] |
| Ileal mucus | 4.81 ± 0.57 | 3.37 ± 0.41 | 75 ± 14 |
| Cecal mucus | 7.15 ± 0.27 | 5.26 ± 0.57 | 83 ± 7.9 |
| Colonic mucus | 6.73 ± 0.27 | 4.63 ± 0.82 | 72 ± 11 |
| Feces | 8.39 ± 0.07 | 6.58 ± 0.19 | 76 ± 13 |

[a] The values are means for three mice. Mucus preparations were isolated on day 15 postfeeding. The CFU values for the mucus preparations are corrected for the entire volume of each mucus preparation. The fecal values are the CFU/g of feces at 15 days after feeding.

[b] A total of 50 *E. coli* MG1655 colonies isolated from the 15-day mucuspreparations and feces of each of the three mice were tested for motility asdescribed in Materials and Methods. The values are means for three mice.

**TABLE 3** Motility of *E. coli* MG1655 strains

| *E. coli* strain | Mean spread (mm) ± SEM (*P*)[a] | |
|---|---|---|
| | Expt 1 | Expt 2 |
| MG1655 | 8.3 ± 0.4 | 9.6 ± 0.2 |
| MG1655 Δ*flhD* | 0 | 0 |
| MG1655-IS*1* | 1.8 ± 0.4 (<0.005) | 1.6 ± 0.3 (<0.001) |
| MG1655 mot-1 | 3.7 ± 0.4 (<0.005) | 3.1 ± 0.4 (<0.002) |
| MG1655 mot-1 restored to wild type | ND | 8.8 ± 0.3 (>0.05) |
| MG1655 mot-2 | 3.7 ± 0.7 (0.005) | ND |
| MG1655 mot-3 | 4.4 ± 0.8 (<0.01) | ND |

[a] That is, the spread of *E. coli* MG1655 strains on Luria motility agar containing 200 mM NaCl after 6 h at 37°C as described in Materials and Methods. Values represent the means of three samples. *P* values, determined using the Student *t* test and indicated in parentheses, were calculated for each strain relative to *E. coli* MG1655 in the same experiment. ND, not done.

strains were designated *E. coli* MG1655 mot-1, mot-2, and mot-3, respectively.

***E. coli* MG1655 mot-1, mot-2, and mot-3 are *envZ* missense mutants.** *E. coli* MG1655 mot-1, mot-2, and mot-3 were less motile than the hypermotile *E. coli* MG1655 (Table 3). Previously, we reported that when the IS*1* element was removed from the *flhDC* regulatory region of *E. coli* MG1655, the resulting strain, *E. coli* MG1655-IS*1*, was a better colonizer and remained motile, although far less so than its parent (24). However, *E. coli* MG1655 mot-1, mot-2, and mot-3 retained the IS*1* element, as determined by PCR (data not shown).

Giraud et al. reported that when germfree mice were fed *E. coli* MG1655, the mouse intestine selected better-colonizing, less-motile *E. coli* MG1655 mutants that contained point mutations in the *envZ-ompR* locus (25). EnvZ, a histidine kinase, and OmpR, its cognate response regulator, comprise a two-component signal transduction system that modulates gene expression in response to osmolarity (17). When we sequenced the *envZ* and *ompR* genes of the three mot mutants—*E. coli* MG1655 Δ*flhD*, *E. coli* MG1655-IS*1*, and *E. coli* MG1655—we found that the *ompR* sequences of the six strains were identical, as were the *envZ* sequences of *E. coli* MG1655 Δ*flhD*, *E. coli* MG165-IS*1*, and the wild-type *E. coli* MG1655. Importantly, *E. coli* MG1655 mot-1, mot-2, and mot-3 were found to be *envZ* missense mutants: mot-1 a P41L mutant (CCG to CTG) in the N-terminal transmembrane domain, mot-2 a P148S mutant (CCG to TCG) in the periplasmic osmolarity sensing domain, and mot-3 a V33E mutant (GTG to GAG) in the N-terminal transmembrane domain (21, 69). That the *envZ* missense mutation in mot-1 was responsible for reduced motility was shown by the fact that when *E. coli* MG1655 mot-1 was restored to the wild type with respect to *envZ*, it regained hypermotility (Table 3).

**The *E. coli* mot mutants are resistant to bile salts and colicin V.** Among the many activities controlled by the two component EnvZ/OmpR signal transduction system are transcription of *ompF* and *ompC*, which encode *E. coli* outer membrane porins that allow passage of many small molecules (≤600 Da) from the environment into the periplasm (1). Bile salts, which are present in the human small intestine at a concentration of ca. 0.3% (64) and can inhibit *E. coli* growth at 5% concentration *in vitro* (25), pass readily through the OmpF porin but much less so through the OmpC porin (62). High levels of OmpR~P downregulate tran-

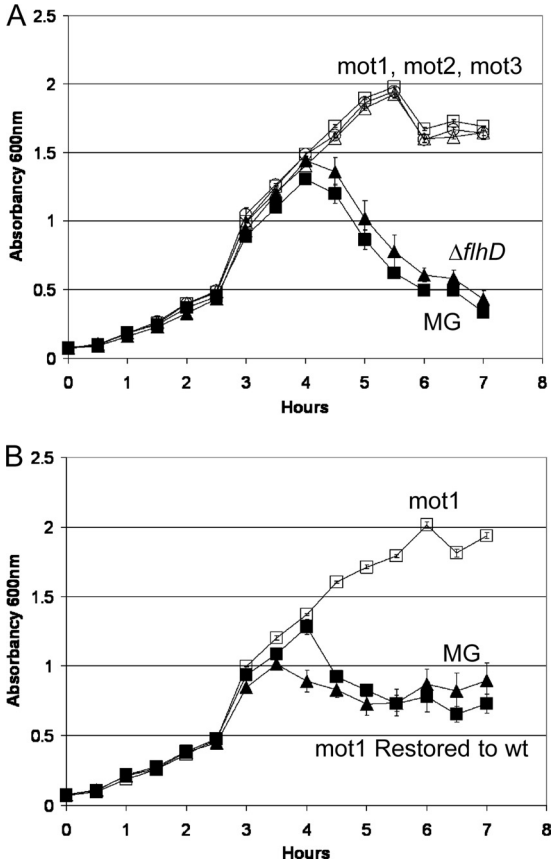


**FIG 2** Growth of *E. coli* MG1655 strains in the presence of 5% bile salts. (A and B) *E. coli* MG1655 Str$^r$ (■), *E. coli* MG1655 mot-1 Str$^r$ (□), *E. coli* MG1655 mot-2 Str$^r$ (○), *E. coli* MG1655 mot-3 Str$^r$ (△), and *E. coli* MG1655 Str$^r$ Δ*flhD*::*cat* (▲) (A) and *E. coli* MG1655 Str$^r$ (▲), *E. coli* MG1655 mot-1 Str$^r$ (□), and *E. coli* MG1655 mot-1 Str$^r$ restored to wild type (■) (B) in Luria broth Lennox containing 5% bile salts (wt/vol). 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.

scription of *ompF* and upregulate transcription of *ompC*, resulting in decreased OmpF and increased OmpC in the outer membrane (55). The *E. coli* MG1655 *envZ* missense mutants described by Girard et al. as being selected in germfree mice monoassociated with *E. coli* MG1655 were reported to have decreased OmpF and increased OmpC and were found to be more resistant to 5% bile salts than their parent (25), suggesting that the *envZ* mutants had higher levels of OmpR∼P than *E. coli* MG1655. Here, we report that *E. coli* MG1655 mot-1, mot-2, and mot-3 are far more resistant to 5% bile salts than *E. coli* MG1655, *E. coli* MG1655 Δ*flhD*, and an *E. coli* MG1655 mot-1 strain restored to wild type (Fig. 2A and 2B). It should also be noted that the *E. coli* MG1655 and *E. coli* MG1655 Δ*flhD* strains are still relatively resistant to bile salts, i.e., their growth is unaffected by 0.3% bile salts (data not shown), the concentration reported to be present in the human small intestine (64).

In addition to regulating *ompF* and *ompC*, the EnvZ/OmpR signal transduction system also positively regulates the transcription of *omrA* and *omrB*, which encode two small RNAs that negatively regulate the expression of a number of outer membrane proteins, including CirA, the receptor for colicin V (7). Since it appeared likely that the *E. coli* MG1655 mot mutants have higher than normal levels of OmpR∼P, it seemed possible that they would have lower levels of CirA in the outer membrane and therefore be more resistant to the action of colicin V. Indeed, *E. coli* F-18, which produces colicin V (44), inhibited the growth of *E. coli* MG1655 (zone of inhibition, 2.01 ± 0.11 mm [$n = 8$]), *E. coli* MG1655 Δ*flhD* (zone of inhibition, 2.03 ± 0.11 mm [$n = 8$]), and *E. coli* MG1655 mot-1 restored to wild type (zone of inhibition, 1.98 ± 0.21 mm [$n = 8$]) to a greater extent than it inhibited the growth of *E. coli* MG1655 mot-1 (zone of inhibition, 0.40 ± 0.07 mm [$n = 8$]), *E. coli* MG1655 mot-2 (zone of inhibition, 0.53 ± 0.13 mm [$n = 8$]), and *E. coli* MG1655 mot-3 (zone of inhibition, 0.31 ± 0.08 mm [$n = 8$]) ($P < 0.0005$ in each case), suggesting that the mot mutants have less-than-normal levels of CirA in their outer membranes. Furthermore, since *omrA* and *omrB* also negatively regulate expression of a number of other outer membrane proteins (26), such as CsgD (regulator of Curli biosynthesis), it is likely that the outer membranes of the *E. coli* MG1655 mot mutants differ markedly from those of *E. coli* MG1655 and *E. coli* MG1655 Δ*flhD*.

***E. coli* MG1655 mot-1 is a better mouse intestinal colonizer than *E. coli* MG1655 and equal to *E. coli* MG1655 Δ*flhD*.** The P41L replacement in EnvZ previously was shown to result in higher than normal levels of OmpR∼P in *E. coli* AT142 (63). We therefore chose to study *E. coli* MG1655 mot-1 in more detail since it has the same *envZ* missense mutation. We found that when $10^5$ CFU each of *E. coli* MG1655 mot-1 and *E. coli* MG1655 were fed to mice simultaneously, *E. coli* MG1655 mot-1 colonized at a level of $\sim 10^9$ CFU per g of feces, whereas *E. coli* MG1655 colonized at a level of between $10^5$ and $10^6$ CFU per g of feces (Fig. 3A). The enhanced colonization of *E. coli* MG1655 mot-1 could be specifically attributed to the P41L replacement in EnvZ, since *E. coli* MG1655 mot-1 restored to wild type and *E. coli* MG1655 cocolonized at equal population sizes (data not shown). Moreover, *E. coli* MG1655 mot-1 was as good a colonizer as *E. coli* MG1655 Δ*flhD* (Fig. 3B). Therefore, *E. coli* MG1655 mot-1 is a far better colonizer than *E. coli* MG1655 wild type and as competitive as *E. coli* MG1655 Δ*flhD*, which explains why the mot-1 strain can remain in the intestine at high levels despite conversion of the majority of the wild-type *E. coli* MG1655 to the Δ*flhD* genotype with its superior colonization phenotype.

**Growth of *E. coli* MG1655 mot-1 on sugars *in vitro*.** To address the question of whether *E. coli* MG1655 mot-1 and *E. coli* MG1655 Δ*flhD* are equally good colonizers because they grow equally well on sugars present in the mouse intestine, experiments were performed to compare the growth rates of *E. coli* MG1655 mot-1, *E. coli* MG1655 Δ*flhD*, and *E. coli* MG1655 on a variety of sugars as the sole source of carbon and energy. *E. coli* MG1655 mot-1 and *E. coli* MG1655 Δ*flhD* did not grow equally well on all of the sugars tested. In fact, *E. coli* MG1655 mot-1 grew 30% faster than *E. coli* MG1655 Δ*flhD* on galactose ($P < 0.0005$) and 5% faster on *N*-acetylglucosamine ($P < 0.01$) but grew slower than *E. coli* MG1655 Δ*flhD* on arabinose (15%, $P < 0.005$), fucose (14%, $P < 0.01$), glucose (10%, $P < 0.01$), maltose (27%, $P < 0.001$), and mannose (5%, $P < 0.025$) and at the same rate as *E. coli* MG1655 Δ*flhD* on fructose ($P = 0.15$), gluconate ($P > 0.2$), and ribose ($P > 0.05$) (Table 4). Also, *E. coli* MG1655 mot-1 grew faster than *E. coli* MG1655 on fructose (16%, $P < 0.01$), galactose (34%, $P < 0.0005$), gluconate (11%, $P < 0.005$), mannose (9%, $P < 0.05$), and *N*-acetylglucosamine (21%, $P < 0.001$), slower than *E. coli*

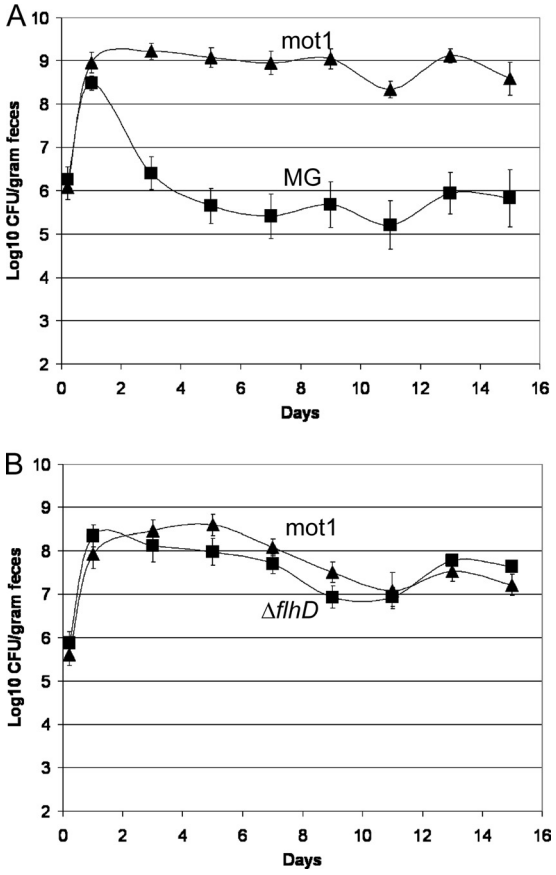


**FIG 3** Colonization of the mouse intestine by *E. coli* MG1655, *E. coli* MG1655 mot-1, *E. coli* MG1655 mot-1 restored to wild type, and *E. coli* MG1655 Δ*flhD*. Three sets of three mice were fed $10^5$ CFU of MG1655 mot-1 $Str^r$ (▲) and $10^5$ CFU of *E. coli* MG1655 $Str^r$ $Nal^r$ (■) (A), and three sets of three mice were fed $10^5$ CFU of *E. coli* MG1655 mot-1 $Str^r$ (▲) and $10^5$ CFU of *E. coli* MG1655 $Str^r$ Δ*flhD*::*cat* (■) (B). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. When necessary, i.e., to distinguish strains, 100 colonies from plates containing streptomycin were toothpicked onto MacConkey agar plates containing streptomycin and nalidixic acid or onto MacConkey agar plates containing streptomycin and chloramphenicol. Bars represent standard errors of the $\log_{10}$ means of CFU per g of feces.

MG1655 on maltose (12%, $P < 0.005$), and at the same rate as *E. coli* MG1655 on arabinose ($P > 0.10$), fucose ($P > 0.10$), glucose ($P > 0.05$), and ribose ($P > 0.05$) (Table 4). In addition, *E. coli* MG1655 Δ*flhD* grew 4 to18% faster than *E. coli* MG1655 on all of the sugars tested as the sole carbon and energy sources ($P < 0.01$ to $< 0.0005$ depending on the sugar) except for galactose, on which the two strains grew equally well ($P > 0.15$) (Table 4). That the 34% faster growth rate of *E. coli* MG1655 mot-1 on galactose relative to wild-type *E. coli* MG1655 was due to the P41L mutation in EnvZ was shown by the fact that the growth rates of *E. coli* MG1655 mot-1 restored to wild type and *E. coli* MG1655 on galactose were identical ($P > 0.15$) (Table 4).

**Growth of *E. coli* MG1655 mot-1 in cecal mucus *in vitro*.** *E. coli* MG1655 simultaneously metabolizes a number of sugars when it colonizes the streptomycin-treated mouse intestine (8, 19). That *E. coli* MG1655 mot-1 grew slower than *E. coli* MG1655 Δ*flhD* on arabinose, fucose, and maltose, three sugars used by *E. coli* MG1655 for growth in the mouse intestine (8, 19, 33), but faster on galactose and *N*-acetylglucosamine could explain why *E. coli* MG1655 mot-1 colonizes mice as well as *E. coli* MG1655 Δ*flhD*. Since *E. coli* colonizes the intestine by growing in intestinal mucus (39, 45, 46, 48, 59, 60, 65), which contains at least 13 different monosaccharides that can be used by *E. coli*, we examined the growth rates of *E. coli* MG1655 mot-1, the *E. coli* MG1655 mot-1 strain restored to wild type, *E. coli* MG1655, and *E. coli* MG1655 Δ*flhD* in mouse cecal mucus standing cultures (2.5 mg/ml with respect to protein). Under these conditions, *E. coli* MG1655 mot-1 grew ca. 15% slower than *E. coli* MG1655 Δ*flhD* ($P < 0.025$) but at about the same rate as *E. coli* MG1655 ($P > 0.15$) and *E. coli* MG1655 mot-1 restored to wild type ($P = 0.50$) (Table 5). Therefore, the differences in growth rates of the strains in cecal mucus do not reflect their relative colonizing abilities, i.e., *E. coli* MG1655 mot-1 = *E. coli* MG1655 Δ*flhD* > *E. coli* MG1655 (Fig. 3).

***E. coli* MG1655 mot-1 resides in all intestinal niches occupied by *E. coli* MG1655 Δ*flhD*.** Although low numbers of *E. coli* MG1655 Δ*flhD* grew to higher numbers in the presence of high numbers of *E. coli* MG1655 in the mouse intestine (Fig. 1), it did not grow up relative to *E. coli* MG1655 mot-1, i.e., the two strains, fed to mice in numbers 5 orders of magnitude apart, remained 5 to

**TABLE 4** Growth of MG1655 strains on various carbon sources[a]

| Carbon source | Mean generation time (min) ± SEM ($n = 3$)[b] | | | | |
|---|---|---|---|---|---|
| | MG1655 | MG1655 mot-1 | MG1655 Δ*flhD* | MG1655 mot-1 restored to wild type | MG1655 mot-1 Δ*galK* |
| Arabinose | 98 ± 1.0 | 97 ± 0.3 | 83 ± 1.2 | ND | ND |
| Fucose | 115 ± 3.1 | 118 ± 1.2 | 102 ± 2.9 | ND | ND |
| Fructose | 139 ± 1.8 | 117 ± 3.5 | 114 ± 1.2 | ND | ND |
| Galactose | 204 ± 3.5 | 135 ± 1.2 | 201 ± 1.2 | 207 ± 1.1 | NG |
| Gluconate | 87 ± 0.7 | 77 ± 1.3 | 76 ± 0.3 | ND | ND |
| Glucose | 83 ± 1.2 | 86 ± 1.8 | 77 ± 0.9 | ND | ND |
| Maltose | 112 ± 2.0 | 127 ± 1.6 | 101 ± 0.2 | ND | ND |
| Mannose | 192 ± 4.2 | 175 ± 2.9 | 166 ± 0.3 | ND | ND |
| NAG[c] | 107 ± 1.5 | 85 ± 0.4 | 89 ± 0.8 | 105 ± 1.3 | ND |
| Ribose | 181 ± 1.2 | 179 ± 0.3 | 174 ± 1.3 | ND | ND |

[a] Carbon source utilization was determined in M9 minimal medium (0.4% [wt/vol]) in 125-ml tissue culture flasks with shaking. Inocula were grown on M9 glycerol (0.4% [vol/vol]) and then overnight on the specified carbon source.
[b] Generation times were calculated from semilogarithmic plots. ND, not done; NG, no growth.
[c] NAG, *N*-acetylglucosamine.

**TABLE 5** Growth of MG1655 strains in cecal mucus *in vitro*[a]

| Carbon source (cecal mucus sample) | Mean rate of growth ($\Delta A_{600}$/h) ± SD[b] | | | | |
|---|---|---|---|---|---|
| | MG1655 | MG1655 mot-1 | MG1655 Δ*flhD* | MG1655 restored to wild type | MG1655 mot-1 Δ*galK* |
| 1 | 0.037 ± 0.002 | 0.038 ± 0.0006 | 0.045 ± 0.0006 | 0.037 ± 0.003 | ND |
| 2 | ND | 0.032 ± 0.002 | 0.037 ± 0.002 | ND | 0.028 ± 0.001 |

[a] Cultures were grown overnight in LB broth Lennox, washed in HEPES-Hanks buffer (pH 7.0), used to inoculate 1 ml of cecal mucus (2.5 mg/ml) in HEPES-Hanks buffer, and then incubated at 37°C in polystyrene cuvettes. The $A_{600}$ of each culture was determined at 30-min intervals. The results of independent experiments ($n = 3$) are shown.
[b] Cecal mucus cultures grew linearly. The slope of the line was used to measure the growth rate.

6 orders of magnitude apart throughout the 15 days of the experiment (Fig. 4A). *E. coli* MG1655 mot-1 did not prevent low numbers of *E. coli* MG1655 Δ*flhD* from growing to higher numbers due to *flhDC* deletion mutants arising in the *E. coli* MG1655 mot-1 population, since at 7 and 15 days postfeeding 50 *E. coli* MG1655 mot-1 colonies from each mouse were tested, and all remained motile. Nor was it due to an inhibitor made by *E. coli* MG1655, since *E. coli* MG1655 Δ*flhD* growth was unaffected by *E. coli* MG1655 mot-1 in colicin tests (data not shown). Clearly, the enhanced colonization of *E. coli* MG1655 mot-1 was due specifically to the P41L *envZ* mutation, since *E. coli* MG1655 Δ*flhD* grew from low to high numbers in the mouse intestine in the presence of high numbers of *E. coli* MG1655 mot-1 restored to wild type (Fig. 4B). Therefore, it is likely that high numbers of *E. coli* MG1655 mot-1 prevented low numbers of *E. coli* MG1655 Δ*flhD* from growing to higher numbers because *E. coli* MG1655 mot-1 resides in and competes effectively in all intestinal niches occupied by *E. coli* MG1655 Δ*flhD*.

*E. coli* MG1655 does not use galactose to colonize the streptomycin-treated mouse intestine, i.e., an *E. coli* MG1655 Δ*galK* mutant that is unable to grow on galactose as a sole carbon source cocolonizes equally well with *E. coli* MG1655 wild type in the streptomycin-treated mouse intestine (19). However, it appears that the ability of *E. coli* MG1655 mot-1 to grow 30% faster on galactose than *E. coli* MG1655 Δ*flhD* contributes in part to it preventing low numbers of *E. coli* MG1655 Δ*flhD* from growing to higher numbers. When *E. coli* MG1655 Δ*flhD* was fed to mice at 5 orders of magnitude lower than *E. coli* MG1655 mot-1 Δ*galK*, it colonized at a level 4 orders of magnitude lower (Fig. 4C), rather than the 5 to 6 orders of magnitude lower in competition with *E. coli* MG1655 mot-1 (Fig. 4A) or *E. coli* MG1655 mot-1 Δ*galK* restored to wild type with respect to *galK* (data not shown). It should be emphasized, however, that *E. coli* MG1655 mot-1 Δ*galK* still prevented *E. coli* MG1655 Δ*flhD* from growing to a level any higher than 4 orders of magnitude below it, despite growing 25% slower than *E. coli* MG1655 mot-1 in cecal mucus *in vitro* ($P < 0.01$) (Table 5).

***E. coli* MG1655 mot-1 may colonize an intestinal niche that *E. coli* MG1655 Δ*flhD* colonizes poorly.** That *E. coli* MG1655 mot-1 resides in all intestinal niches occupied by *E. coli* MG1655 Δ*flhD* does not mean *E. coli* MG1655 Δ*flhD* resides in all intestinal niches occupied by *E. coli* MG1655 mot-1. Since *E. coli* MG1655 mot-1 and *E. coli* MG1655 Δ*flhD* are equally good colonizers when $10^5$ CFU of each is fed to mice (Fig. 3C) and since high numbers of *E. coli* MG1655 mot-1 prevent low numbers of *E. coli* MG1655 Δ*flhD* from growing to high numbers in the intestinal niches they occupy together (Fig. 4A), it would be expected that if *E. coli* MG1655 Δ*flhD* resides in all intestinal niches occupied by *E. coli* MG1655 mot-1 that high numbers of *E. coli* MG1655 Δ*flhD* would prevent low numbers of *E. coli* MG1655 mot-1 from growing to higher numbers in the streptomycin-treated mouse intestine. To test that possibility, mice were fed $10^{10}$ CFU of *E. coli* MG1655 Δ*flhD* and $10^5$ CFU of *E. coli* MG1655 mot-1. Surprisingly, *E. coli* MG1655 mot-1 grew from about 5 orders of magnitude lower than *E. coli* MG1655 Δ*flhD* to within 2 orders of magnitude of *E. coli* MG1655 Δ*flhD* by 9 days postfeeding (Fig. 5A). Therefore, it appears that *E. coli* MG1655 mot-1 not only resides in all the intestinal niches occupied by *E. coli* MG1655 Δ*flhD* but may also colonize a smaller niche that *E. coli* MG1655 Δ*flhD* is unable to colonize or colonizes poorly. Moreover, it appears that *E. coli* MG1655 mot-1 uses its superior ability to metabolize galactose to colonize that niche, since low numbers of *E. coli* MG1655 mot-1 Δ*galK* failed to grow to higher numbers in the presence of high numbers of *E. coli* MG1655 Δ*flhD* (Fig. 5B), whereas low numbers of *E. coli* MG1655 mot-1 Δ*galK* restored to wild type with respect to galactose utilization grew to higher numbers (data not shown). To summarize, *E. coli* MG1655 mot-1 and *E. coli* MG1655 Δ*flhD* appear to colonize one intestinal niche equally well and *E. coli* MG1655 mot-1 appears to need galactose for maximum growth in that niche. In addition, it appears that *E. coli* MG1655 mot-1 also uses galactose to colonize a second smaller intestinal niche that *E. coli* MG1655 Δ*flhD* either fails to colonize or colonizes poorly.

***E. coli* MG1655 forms mixed biofilms in the mouse intestine.** Commensal *E. coli* strains colonize the streptomycin-treated mouse intestine by growing in the intestinal mucus layer of the large intestine (39, 45, 46, 48, 59, 60, 65). It has recently become clear that biofilms form in the mucus layers of the large intestines of humans, rats, baboons, and mice (49, 61) and that mixed biofilms consisting of bacteroides, enterobacter, and clostridium species form rapidly on strands of mucin in mucus introduced into a growing human microbiota contained in a continuous flow culture system constructed to mimic the human intestine (41). Moreover, the kinetics of plasmid transfer between *E. coli* strains in the streptomycin-treated mouse intestine suggests that *E. coli* resides in biofilms *in vivo* (38). Previously, we reported that *E. coli* MG1655 is found in cecal mucus dispersed as either single cells or dividing doublets among other members of the microbiota at 24 h postfeeding (45, 46); however, in view of the recent data on biofilm formation in the mammalian intestine, we wondered whether dense microbial communities containing *E. coli* MG1655 might form in the streptomycin treated mouse intestine at later times. As shown previously and in Fig. 6A, at 24 h postfeeding *E. coli* MG1655 appeared to be randomly dispersed and yet closely associated with other members of the microbiota in the cecal mucus layer; however, at 48 h postfeeding, although *E. coli* MG1655 remained mostly as single cells or as doublets, they appeared to be

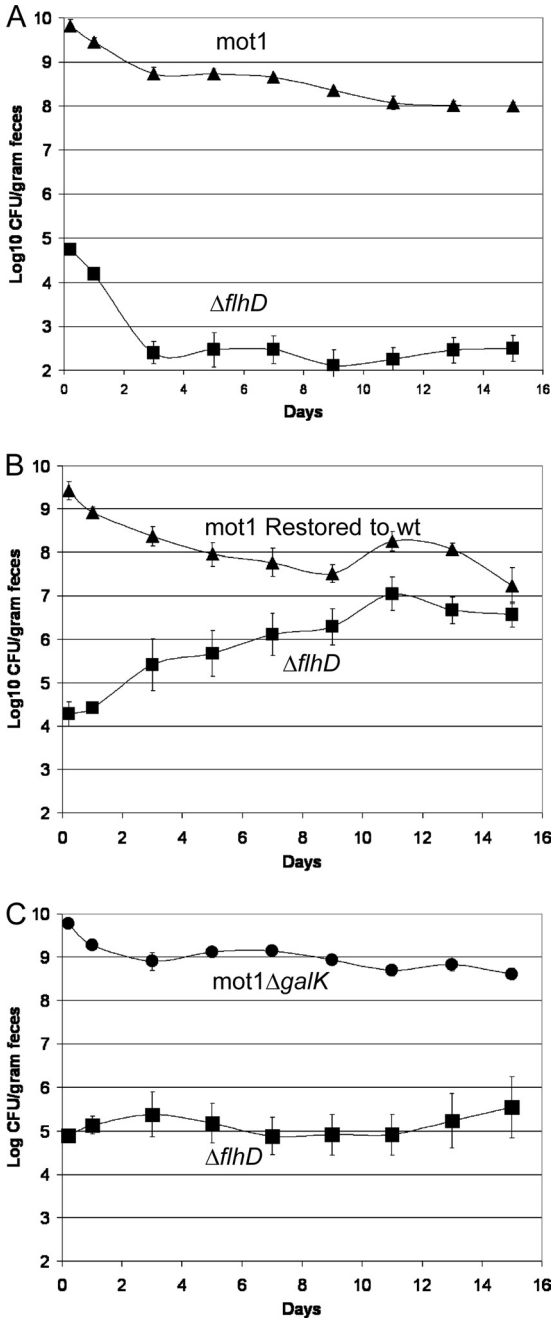


**FIG 4** Colonization of the mouse intestine by *E. coli* MG1655 mot-1, *E. coli* MG1655 mot-1 restored to wild type, *E. coli* MG1655 mot-1 Δ*galK*, and *E. coli* MG1655 Δ*flhD*. Two sets of three mice were fed $10^{10}$ CFU of *E. coli* MG1655 mot-1 Str$^r$ (▲) and $10^5$ CFU of MG1655 Str$^r$ Δ*flhD*::*cat* (■) (A); two sets of three mice were fed $10^{10}$ CFU of *E. coli* MG1655 mot-1 Str$^r$ restored to wild type (▲) and $10^5$ CFU of *E. coli* MG1655 Str$^r$ Δ*flhD*::*cat* (■) (B); and three sets of three mice were fed $10^{10}$ CFU of *E. coli* MG1655 mot-1 Str$^r$ Δ*galK* (●) and $10^5$ CFU of *E. coli* MG1655 Str$^r$ Δ*flhD*::*cat* (■) (C). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. When necessary, i.e., to distinguish strains, 100 colonies from plates containing streptomycin were toothpicked onto MacConkey agar plates containing streptomycin and chloramphenicol. Bars represent standard errors of the $\log_{10}$ means of CFU per g of feces.

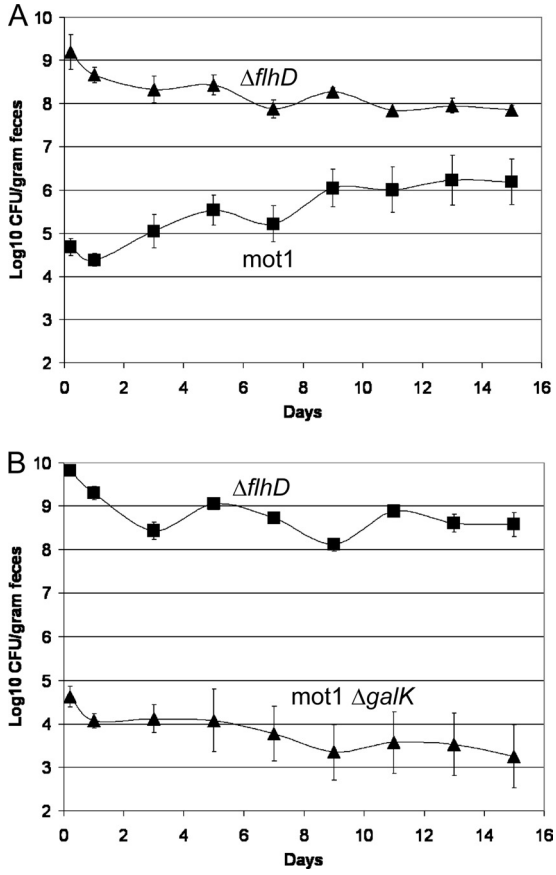


**FIG 5** Colonization of the mouse intestine by *E. coli* MG1655 mot-1, *E. coli* MG1655 mot-1 Δ*galK*, and *E. coli* MG1655 Δ*flhD*. Three sets of three mice were fed $10^{10}$ CFU of *E. coli* MG1655 Str$^r$ Δ*flhD*::*cat* (▲) and $10^5$ CFU of *E. coli* MG1655 mot-1 Str$^r$ Nal$^r$ (■) (A) and $10^{10}$ CFU of *E. coli* MG1655 Str$^r$ Δ*flhD* (■) and $10^5$ CFU of *E. coli* MG1655 mot-1 Str$^r$ Δ*galK*::*cat* (▲) (B). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars represent standard errors of the $\log_{10}$ means of CFU per g of feces.

minority members of dense mixed biofilms (Fig. 6B). At neither 24 h nor 48 h postfeeding was *E. coli* MG1655 found to be associated with the epithelium (Fig. 6).

## DISCUSSION

Previously, we reported that the streptomycin-treated mouse intestine selects nonmotile *E. coli* MG1655 *flhDC* deletion mutants that have improved colonizing ability (24, 36). These mutants grew 15% faster than their parent in mouse cecal mucus *in vitro* and 15 to 30% faster on several sugars found in the mouse intestine, suggesting that their better colonizing ability was due to their more efficient utilization of sugars for growth (36). In our previous studies, despite the strong selection for nonmotile *flhDC* deletion mutants, 10 to 20% of the *E. coli* MG1655 cells remained motile (24). In the present study, we demonstrate that these motile *E. coli* MG1655 contain missense mutations in *envZ* that make them less motile and better colonizers than wild-type *E. coli* MG1655 and more resistant to bile salts and colicin V than *E. coli* MG1655 and *E. coli* MG1655 Δ*flhD*, suggesting that they have higher levels of OmpR~P.

Among the many activities controlled by EnvZ/OmpR is tran-

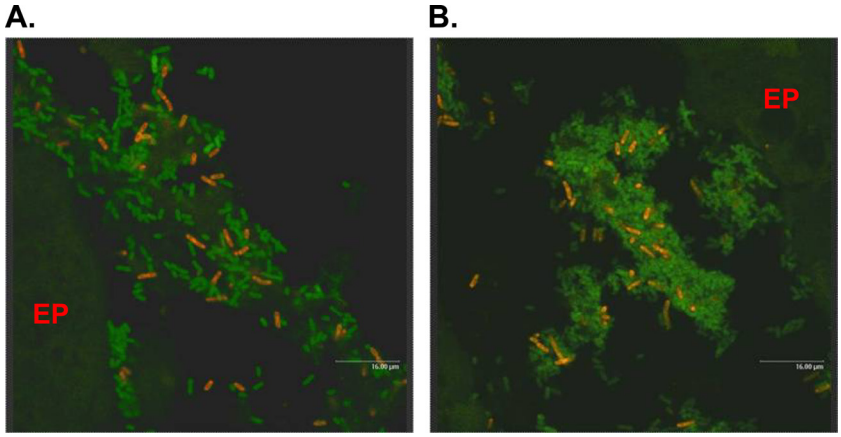


**FIG 6** *In situ* hybridization with fluorescence-labeled oligonucleotide probes. At 24 and 48 h postfeeding, cecal mucosal sections of mice fed *E. coli* MG1655 Str$^r$ were hybridized with an *E. coli*-specific oligonucleotide probe (red) and a eubacterium-specific oligonucleotide probe (green). EP, epithelium. *E. coli* cells appear red, while all other bacteria appear green. Bars, 16 $\mu$m. (A) 24 h postfeeding; (B) 48 h postfeeding.

scription of the *flhDC* operon, i.e., OmpR~P is a negative regulator of the operon in *E. coli* strains that lack the IS*1* element in the *flhDC* promoter (56). Such strains are not hypermotile and become even less motile at higher medium osmolarity because the level of OmpR~P under these conditions is sufficient to further repress the *flhDC* operon (4, 24). In contrast, *E. coli* MG1655, which contains the IS*1* element in the *flhDC* promoter, remains hypermotile under high-osmolarity conditions (4, 24), suggesting the possibility that the IS*1* element reduces the affinity of OmpR~P for the OmpR binding sites in the *flhDC* promoter. The fact that the *E. coli* MG1655 *envZ* missense mutants are far less motile than *E. coli* MG1655 (Table 3) suggests that the even higher level of OmpR~P in these mutants allows some level of binding to the OmpR binding sites in the *flhDC* regulatory region, resulting in less transcription of the operon and consequently less $FlhD_4C_2$, despite the presence of the IS*1* element. Also, since $FlhD_4C_2$ represses a number of sugar catabolism operons (20), less $FlhD_4C_2$ in *E. coli* MG1655 mot-1 than in *E. coli* MG1655 but more than in *E. coli* MG1655 Δ*flhD* would be consistent with it growing faster than *E. coli* MG1655 on a number of sugars and slower than *E. coli* MG1655 Δ*flhD* on a number of sugars, as we observed (Table 4).

Although *E. coli* MG1655 mot-1 grew slower than *E. coli* MG1655 Δ*flhD* on several sugars, it did grow ca. 30% faster than *E. coli* MG1655 Δ*flhD* and *E. coli* MG1655 on galactose *in vitro* (Table 4). In this regard, it is interesting that there is a putative OmpR binding site upstream of the *galR* promoter between *galR* and *omrB* (26). GalR is the major repressor of both galactose transport and catabolism (67). Although it is known that OmpR~P positively regulates transcription of *omrB* (26), it is not known whether it also negatively regulates *galR* transcription. Negative regulation of *galR* transcription by increased levels of OmpR~P in *E. coli* MG1655 mot-1 could explain why it grows faster than *E. coli* MG1655 and *E. coli* MG1655 Δ*flhD* on galactose as the sole source of carbon and energy.

Giraud et al. (25) demonstrated that when germfree mice were fed the same wild-type *E. coli* MG1655 strain as used here, the mouse intestine selected better-colonizing, less-motile *E. coli* MG1655 *envZ* missense mutants. More recently, De Paepe et al. (14) demonstrated that several days after the *envZ* missense mutants reached maximum numbers in germfree mice monoassociated with *E. coli* MG1655, mutants with *flhDC* deletions appeared and increased in numbers thereafter, showing that the monoassociated mouse intestine can select *E. coli* MG1655 *flhDC* deletion mutants in the presence of high numbers of *E. coli* MG1655 *envZ* missense mutants. Interestingly, our data show that the monoassociated ex-germfree mouse intestine and the streptomycin-treated mouse intestine are different in this regard: *flhDC* mutants would not be selected after *envZ* missense mutants reach maximum numbers in the streptomycin-treated mouse intestine since in this model high numbers of an *E. coli* MG1655 *envZ* missense mutant prevented growth of an *E. coli* MG1655 *flhDC* deletion mutant from lower to higher numbers (Fig. 4A).

It was also demonstrated by De Paepe et al. (14) that their *in vivo* results could be reproduced in chemostats containing 0.8% bile salts, a concentration reasonable for the intestine of a germfree mouse (18), but ~2.7-fold higher than the 0.3% concentration of bile salts reported to be in the human small intestine (64). In fact, the presence of a conventional microbiota decreases the concentration of bile salts in the mouse intestine, i.e., in the conventional mouse cecum and colon the concentration of bile salts is known to be ~2.5-fold lower than in the germfree mouse cecum and colon (18). It is therefore unlikely that bile salts play as much of a role in *E. coli* MG1655 colonization of the streptomycin-treated mouse intestine, which contains a large and diverse microbiota, as they do in its colonization of the monoassociated mouse intestine. In support of this view, despite *E. coli* MG1655 Δ*flhD* being more sensitive to bile salts than *E. coli* MG1655 mot-1, the two strains grew equally well from low to high numbers in the streptomycin treated mouse intestine when $10^5$ CFU each of the two strains were fed to mice simultaneously (Fig. 3B). It therefore appears likely that the differences between our results and those of De Paepe et al. (14) reflect physical and nutritional interaction of the *E. coli* MG1655 strains with other members of the complex microbial community present in the streptomycin-treated mouse intestine but absent in the monoassociated, formerly germfree mouse intestine.

The concept that nutrient availability dictates the community structure of the intestine was originally presented in Freter's nutrient-niche hypothesis, which states that species coexist in the intestine because each is able to grow faster than all others on one

or a few limiting nutrients and that the rate of growth of each species during the colonization process is at least equal to its washout rate from the intestine (22). The hypothesis assumes that all nutrients are perfectly mixed and that they are equally available to all species present in the intestine. According to the hypothesis, two strains cannot coexist in the intestine when one competes less well than the other for the same nutrient(s) unless the metabolically less efficient one adheres to the intestinal wall (22). Adhesion of a bacterium to the intestinal wall is not restricted to the epithelium, it could, for example, also be achieved by adhesion of that bacterium to mucin strands in the outer loose mucus layer where commensal strains reside (32, 50) that might be anchored in the relatively bacteria-free inner dense mucus layer or, alternatively by attachment to other members of the microbiota that adhere to mucin strands in the outer loose mucus layer. In either case, such attachment could delay the metabolically less efficient strain from being washed out as the loose mucus layer at the luminal surface is sloughed into luminal contents and eliminated in feces.

We found no evidence that *E. coli* MG1655 adheres to the intestinal epithelium but did find that it binds to other members of the microbiota as a minority member of mixed biofilms in cecal mucus (Fig. 6B). We also found that *E. coli* MG1655 mot-1 and *E. coli* MG1655 Δ*flhD* each use some sugars for growth better than the other *in vitro* (Table 4) and that they cocolonize the mouse intestine equally well when equal numbers of each strain are fed to mice (Fig. 3B), indicating that sufficient levels of the sugars that each strain uses better are available to each strain when they cocolonize. As such, it would be expected according to the nutrient-niche hypothesis that low numbers of each strain would be able to grow to higher numbers in the intestine in the presence of high numbers of the other strain. Indeed, by using its superior ability to grow on galactose, low numbers of *E. coli* MG1655 mot-1 grew to higher numbers in the presence of high numbers of *E. coli* MG1655 Δ*flhD* (Fig. 5A). However, contrary to expectations, high numbers of *E. coli* MG1655 mot-1 prevented low numbers of *E. coli* MG1655 Δ*flhD* from growing to higher numbers (Fig. 4A). Below we outline a modified version of the Freter nutrient-niche hypothesis to explain our results based on the finding that *E. coli* MG1655 appears to reside in mixed biofilms in the streptomycin-treated mouse intestine.

Mono- and disaccharides and maltodextrins are absorbed in the small intestine, whereas dietary fiber reaches the large intestine intact. In contrast to the anaerobes (29), *E. coli* does not secrete extracellular polysaccharide hydrolases (27, 30) and therefore cannot degrade dietary fiber-derived and mucin-derived oligo- and polysaccharides. Commensal *E. coli* strains colonize the mouse large intestine by growing in intestinal mucus (39, 45, 46, 48, 59, 60, 65). It therefore appears likely that *E. coli* depends on the anaerobes present in mucus that can degrade oligo- and polysaccharides to provide them with the mono-and disaccharides they need for growth. We hypothesize that the anaerobes in the mixed biofilms inhabited by *E. coli* provide them with mono- and disaccharides locally, rather than from a perfectly mixed pool available to all species, which is an assumption of the nutrient-niche hypothesis. We further hypothesize that one strain of *E. coli* can have a higher affinity for binding sites on mixed biofilms than a second strain due to differences between the outer surfaces of the two strains. In addition, we hypothesize that *E. coli* cells that detach from a mixed biofilm either spontaneously or through replication and hence free in mucus are not exposed to mono- and disaccharides because those sugars are produced and used locally within the mixed biofilms and are therefore not available to detached *E. coli* cells. Consequently, *E. coli* cells not attached to a mixed biofilm will not grow or will grow slower than the washout rate and will be eliminated in feces unless they bind to and become part of other mixed biofilms that are constantly forming in the mucus layer.

How does the hypothesis account for our results? Our results suggest that the outer membranes of *E. coli* MG1655 mot-1 and *E. coli* MG1655 Δ*flhD* differ not only with respect to the presence or absence of flagella and amounts of OmpF and OmpC but also with respect to the amounts of a number of proteins present in their outer membranes, including CirA and CsgD (see Results and reference 26), which we hypothesize results in *E. coli* MG1655 mot-1 having a higher affinity than *E. coli* MG1655 Δ*flhD* for the *E. coli* binding sites on the mixed biofilms they inhabit. In addition, *E. coli* MG1655 mot-1 grows slower than *E. coli* MG1655 Δ*flhD* on a number of sugars present in mucus and in mouse cecal mucus *in vitro* (Table 5). Accordingly, we suggest that the lower growth rate of *E. coli* MG1655 mot-1 within a biofilm is compensated for by its higher affinity for the biofilm. If so, it would then be expected that if *E. coli* MG1655 mot-1 and *E. coli* MG1655 Δ*flhD* were fed to mice in equal numbers they would cocolonize in about equal numbers, as observed (Fig. 3B), and if mice were fed *E. coli* MG1655 mot-1 and *E. coli* MG1655 Δ*flhD* at a ratio of $10^5$:1, that ratio would also be maintained throughout the duration of the experiment, as observed (Fig. 4A). However, it would also be expected that if mice were fed *E. coli* MG1655 Δ*flhD* and *E. coli* MG1655 mot-1 at a ratio of $10^5$:1, that ratio would be maintained throughout the duration of the experiment, when in fact, under these conditions, low numbers of *E. coli* MG1655 mot-1 grow to higher numbers (Fig. 5A). This can be explained if *E. coli* MG1655 mot-1 also grows in a second niche that *E. coli* MG1655 Δ*flhD* cannot colonize or colonizes poorly. That second niche could be another mixed biofilm consisting of a different group of anaerobes in which *E. coli* MG1655 mot-1 takes advantage not only of its higher affinity for binding sites but also of its superior growth rate on galactose and thereby prevents *E. coli* MG1655 Δ*flhD* from occupying that niche. Alternatively, that second niche could be another mixed biofilm that contains an anaerobe that makes a bacteriocin that inhibits *E. coli* MG1655 Δ*flhD* growth far more than it inhibits *E. coli* MG1655 mot-1 growth, much in the same way that *E. coli* MG1655 Δ*flhD* is more sensitive to colicin V than *E. coli* MG1655 mot-1. Most bacterial species, including several that reside in the mammalian intestine, are known to make bacteriocins, many of which kill across species (54). Finally, our hypothesis raises the possibility that *E. coli* strains with vastly different surfaces (e.g., fimbriae, O, K, and H antigens etc) reside in biofilms made up of different anaerobes that supply different sugars to those strains, which could explain why different *E. coli* strains display different nutritional programs in the intestine (19, 35). Whether our hypothesis, which we call the "Restaurant" hypothesis, has merit will be the focus of future research.

## ACKNOWLEDGMENTS

This research was supported by Public Health Service grants AI48945 and GM095370 to T.C. and P.S.C.

## REFERENCES


1. **Aiba H, Mizuno T.** 1990. Phosphorylation of a bacterial activator protein, OmpR, by a protein kinase, EnvZ, stimulates the transcription of the *ompF* and *ompC* genes in *Escherichia coli.* FEBS Lett. **261**:19–22.
2. **Bachmann BJ.** 1996. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12, p 2460–2488. *In* Neidhardt FC, et al (ed), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, 2nd ed, **vol 2**. American Society for Microbiology, Washington, DC.
3. **Banwell JG, Gorbach SL, Pierce NF, Mitra R, Mondal A.** 1971. Acute undifferentiated human diarrhea in the tropics. II. Alterations in intestinal fluid and electrolyte movements. J. Clin. Invest. **50**:890–900.
4. **Barker CS, Prüss BM, Matsumura P.** 2004. Increased motility of *Escherichia coli* by insertion sequence element integration into the regulatory region of the *flhD* operon. J. Bacteriol. **186**:7529–7537.
5. **Bartlett DH, Frantz BB, Matsumura P.** 1988. Flagellar transcriptional activators FlbB and FlaI: gene sequences and 5′ consensus sequences of operons under FlbB and FlaI control. J. Bacteriol. **170**:1575–1581.
6. **Blattner FR, et al.** 1997. The complete genome sequence of *Escherichia coli* K-12. Science **277**:1453–1474.
7. **Braun V, Patzer SI, Hantke K.** 2002. Ton-dependent colicins and microcins: modular design and evolution. Biochimie **84**:365–380.
8. **Chang D-E, et al.** 2004. Carbon nutrition of *Escherichia coli* in the mouse intestine. Proc. Natl. Acad. Sci. U. S. A. **101**:7427–7432.
9. **Cohen PS, Laux DC.** 1995. Bacterial adhesion to and penetration of intestinal mucus *in vitro.* Methods Enzymol. **253**:309–315.
10. **Conlan JW, Bardy SL, KuoLee R, Webb A, Perry MB.** 2001. Ability of *Escherichia coli* O157:H7 isolates to colonize the intestinal tract of conventional adult CD1 mice is transient. Can. J. Microbiol. **47**:91–95.
11. **Conway T, Krogfelt KA, Cohen PS.** 29 December 2004, posting date. Chapter 8.3.1.2. The life of commensal *Escherichia coli* in the mammalian intestine. *In*Curtiss R, III, et al. (ed), EcoSal—*Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC. http://www.ecosal.org.
12. **Croswell A, Amir E, Teggatz P, Barman M, Salzman NH.** 2009. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric *Salmonella* infection. Infect. Immun. **77**:2741–2753.
13. **Datsenko KA, Wanner BL.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. U. S. A. **97**:6640–6645.
14. **De Paepe M, et al.** 2011. Trade-off between bile resistance and nutritional competence drives *Escherichia coli* diversification in the mouse gut. PLoS Genet. **7**:e1002107.
15. **Dethlefsen L, Huse S, Sogin ML, Relman DA.** 2008. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. **6**:2383–2400.
16. **Eckburg PB, et al.** 2005. Diversity of the human intestinal microbial flora. Science **308**:1635–1638.
17. **Egger LA, Park H, Inouye M.** 1997. Signal transduction via the histidyl-aspartyl phosphorelay. Genes Cells **2**:167–184.
18. **Eyssen HJ, Parmentier GG, Mertens JA.** 1976. Sulfated bile acids in germ-free and conventional mice. Eur. J. Biochem. **66**:507–514.
19. **Fabich AJ, et al.** 2008. Comparison of carbon nutrition for pathogenic and commensal *Escherichia coli* strains in the mouse intestine. Infect. Immun. **76**:1143–1152.
20. **Fabich AJ, et al.** 2011. Genotype and phenotypes of an intestine-adapted *Escherichia coli* K-12 mutant selected by animal passage for superior colonization. Infect. Immun. **79**:2430–2439.
21. **Forst S, Comeau D, Norioka SM.** 1987. Localization and membrane topology of EnvZ, a protein involved in osmoregulation of OmpF and OmpC in *Escherichia coli.* J. Biol. Chem. **262**:16433–16438.
22. **Freter R, Brickner H, Fekete J, Vickerman MM, Carey KE.** 1983. Survival and implantation of *Escherichia coli* in the intestinal tract. Infect. Immun. **39**:686–703.
23. **Garner CD, et al.** 2009. Perturbation of the small intestine microbial ecology by streptomycin alters pathology in a *Salmonella enterica* serovar Typhimurium murine model of infection, Infect. Immun. **77**:2691–2702.
24. **Gauger EJ, et al.** 2007. Role of motility and the *flhDC* operon in *Escherichia coli* MG165 colonization of the mouse intestine. Infect. Immun. **75**:3315–3324.
25. **Giraud A, et al.** 2008. Dissecting the genetic components of adaptation of *Escherichia coli* to the mouse gut. PLoS Genet. **4**:52–61.
26. **Guillier M, Gottesman S.** 2006. Remodeling of the *Escherichia coli* outer membrane by two small regulatory RNAs. Mol. Microbiol. **59**:231–247.
27. **Henrissat B, Davies G.** 1997. Structural and sequence-based classification of glycoside hydrolases. Curr. Opin. Struct. Biol. **7**:637–644.
28. **Hentges DJ, Que JU, Casey SW, Stein AJ.** 1984. The influence of streptomycin on colonization in mice. Microecol. Ther. **14**:53–62.
29. **Hoskins L.** 1984. Mucin degradation by enteric bacteria: ecological aspects and implications for bacterial attachment to gut mucosa, p 51–65. *In* Boedecker EC (ed), Attachment of organisms to the gut mucosa, vol II. CRC Press, Inc, Boca Raton, FL.
30. **Hoskins LC, et al.** 1985. Mucin degradation in human colon ecosystems. Isolation and properties of fecal strains that degrade ABH blood group antigens and oligosaccharides from mucin glycoproteins. J. Clin. Invest. **75**:944–953.
31. **Jiang Y, Pogliano J, Helinski DR, Konieczny I.** 2002. ParE toxin encoded by the broad-host-range plasmid RK2 is an inhibitor of *Escherichia coli* gyrase. Mol. Microbiol. **44**:971–979.
32. **Johansson ME, et al.** 2008. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc. Natl. Acad. Sci. U. S. A. **105**:15064–15069.
33. **Jones SA, et al.** 2008. Glycogen and maltose utilization by *Escherichia coli* O157:H7 in the mouse intestine. Infect. Immun. **76**:2531–2540.
34. **Laux DC, Cohen PS, Conway T.** 2005. Role of the mucus layer in bacterial colonization of the intestine, p 199–212. *In* Nataro JP, et al (ed), Colonization of mucosal surfaces. ASM Press, Washington, DC.
35. **Leatham MP, et al.** 2009. Precolonized human commensal *Escherichia coli* strains serve as a barrier to *E. coli* O157:H7 growth in the streptomycin-treated mouse intestine. Infect. Immun. **77**:2876–2886.
36. **Leatham MP, et al.** 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.
37. **Ley RE, et al.** 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. U. S. A. **102**:11070–11075.
38. **Licht TR, Christensen BB, Krogfelt KA, Molin S.** 1999. Plasmid transfer in the animal intestine and other dynamic bacterial populations: the role of community structure and environment. Microbiology **145**:2615–2622.
39. **Licht TR, Tolker-Nielsen T, Holmstrøm K, Krogfelt KA, Molin S.** 1999. Inhibition of *Escherichia coli* precursor 16S rRNA processing by mouse intestinal contents. Environ. Microbiol. **1**:23–32.
40. **Liu X, Matsumura P.** 1994. The FlhD/FlhC complex, a transcriptional activator of the *Escherichia coli* flagellar class II operons. J. Bacteriol. **176**:7345–7351.
41. **Macfarlane S, Woodmansey EJ, Macfarlane GT.** 2005. Colonization of mucin by human intestinal bacteria and establishment of biofilm communities in a two-stage continuous culture system. Appl. Environ. Microbiol. **71**:7483–7492.
42. **McCormick BA, Franklin DP, Laux DC, Cohen PS.** 1989. Type 1 pili are not necessary for colonization of the streptomycin-treated mouse large intestine by type 1-piliated *Escherichia coli* F-18 and *E. coli* K-12. Infect. Immun. **57**:3022–3029.
43. **Miller CP, Bohnhoff M.** 1963. Changes in the mouse's enteric microbiota associated with enhanced susceptibility to *Salmonella* infection following streptomycin-treatment. J. Infect. Dis. **113**:59–66.
44. **Miller JH.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
45. **Miranda RL, et al.** 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.
46. **Møller AK, et al.** 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.
47. **Myhal ML, Laux DC, Cohen PS.** 1982. Relative colonizing abilities of human fecal and K 12 strains of *Escherichia coli* in the large intestines of streptomycin-treated mice. Eur. J. Clin. Microbiol. **1**:186–192.
48. **Newman JV, Kolter R, Laux DC, Cohen PS.** 1994. The role of *leuX* in *Escherichia coli* colonization of the streptomycin-treated mouse large intestine. Microb. Pathog. **17**:301–311.
49. **Palestrant D, et al.** 2004. Microbial biofilms in the gut: visualization by electron microscopy and by acridine orange staining. Ultrastruct. Pathol. **28**:23–27.
50. **Pearson JP, Brownlee IA.** 2005. Structure and function of mucosal surfaces, p 3–16. *In* Nataro JP, et al (ed), Colonization of mucosal surfaces. ASM Press, Washington, DC.

51. **Prüss BM.** 2000. FlhD, a transcriptional regulator in bacteria. Recent Res. Dev. Microbiol. **4**:31–42.
52. **Prüss BM, et al.** 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.
53. **Prüss BM, Liu X, Hendrickson W, Matsumura P.** 2001. FlhD/FlhC-regulated promoters analyzed by gene array and *lacZ* gene fusions. FEMS Microbiol. Lett. **197**:91–97.
54. **Riley MA, Wertz JE.** 2002. Bacteriocins: evolution, ecology, and application. Annu. Rev. Microbiol. **56**:117–137.
55. **Russo FD, Silhavy TJ.** 1991. EnvZ controls the concentration of phosphorylated OmpR to mediate osmoregulation of the porin genes. J. Mol. Biol. **222**:567–580.
56. **Shin S, Park C.** 1995. Modulation of flagellar expression in *Escherichia coli* by acetyl phosphate and the osmoregulator OmpR. J. Bacteriol. **177**:4696–4702.
57. **Stecher B, et al.** 2010 Like will to like: abundances of closely related species can predict susceptibility to intestinal colonization by pathogenic and commensal bacteria. PLoS Pathog. **6**:e1000711.
58. **Stecher B, et al.** 2007. *Salmonella enterica* serovar Typhimurium exploits inflammation to compete with the intestinal microbiota. PLoS Biol. **5**:2177–2189.
59. **Sweeney NJ, et al.** 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.
60. **Sweeney NJ, Laux DC, Cohen PS.** 1996. *Escherichia coli* F-18 and K-12 *eda* mutants do not colonize the streptomycin-treated mouse large intestine. Infect. Immun. **64**:3504–3511.
61. **Swidsinski A, Weber J, Loening-Baucke V, Hale LP, Lochs H.** 2005. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. J. Clin. Microbiol. **43**:3380–3389.
62. **Thanassi DG, Cheng LW, Nikaido H.** 1997. Active efflux of bile salts by *Escherichia coli.* J. Bacteriol. **179**:2512–2518.
63. **Tokishita S-I, Mizuno T.** 1994. Transmembrane signal transduction by the *Escherichia coli* osmotic sensor, EnvZ: intermolecular complementation of transmembrane signaling. Mol. Microbiol. **13**:435–444.
64. **Van Deest BW, Fordtran JS, Morawski SG, Wilson JD.** 1968. Bile salt and micellar fat concentration in proximal small bowel contents of ileectomy patients. J. Clin. Invest. **47**:1314–1324.
65. **Wadolkowski EA, Laux DC, Cohen PS.** 1988. Colonization of the streptomycin-treated mouse large intestine by a human fecal *Escherichia coli* strain: role of growth in mucus.
66. **Wang S, Fleming RT, Westbrook EM, Matsumura P, McKay DB.** 2006. Structure of the *Escherichia coli* FlhDC complex, a prokaryotic heteromeric regulator of transcription. J. Mol. Biol. **355**:798–808.
67. **Weickert MJ, Adhya S.** 1993. The galactose regulon of *Escherichia coli.* Mol. Microbiol. **10**:245–251.
68. **Wlodarska M, et al.** 2011. Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated *Citrobacter rodentium*-induced colitis. Infect. Immun. **79**:1536–1545.
69. **Yaku H, Mizuno T.** 1997. The membrane-located osmosensory kinase, EnvZ, that contains a leucine zipper-like motif functions as a dimer in *Escherichia coli.* FEBS Lett. **417**:409–413.