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Mouse Intestine Selects Nonmotile *flhDC* Mutants of *Escherichia coli* MG1655 with Increased Colonizing Ability and Better Utilization of Carbon Sources

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D-Gluconate which is primarily catabolized via the Entner-Doudoroff (ED) pathway, has been implicated as being important for colonization of the streptomycin-treated mouse large intestine by *Escherichia coli* MG1655, a human commensal strain. In the present study, we report that an MG1655 Δ*edd* mutant defective in the ED pathway grows poorly not only on gluconate as a sole carbon source but on a number of other sugars previously implicated as being important for colonization, including L-fucose, D-gluconate, D-glucuronate, *N*-acetyl-D-glucosamine, D-mannose, and D-ribose. Furthermore, we show that the mouse intestine selects mutants of MG1655 Δ*edd* and wild-type MG1655 that have improved mouse intestine-colonizing ability and grow 15 to 30% faster on the aforementioned sugars. The mutants of MG1655 Δ*edd* and wild-type MG1655 selected by the intestine are shown to be nonmotile and to have deletions in the *flhDC* operon, which encodes the master regulator of flagellar biosynthesis. Finally, we show that Δ*flhDC* mutants of wild-type MG1655 and MG1655 Δ*edd* constructed in the laboratory act identically to those selected by the intestine; i.e., they grow better than their respective parents on sugars as sole carbon sources and are better colonizers of the mouse intestine.

Bacterial colonization of the intestine is defined as the indefinite persistence of a bacterial population in stable numbers in an animal's intestine without repeated introduction of the bacterium into that animal. Persistence in the intestine is reflected by persistence in feces. Colonization resistance refers to the ability of a complete intestinal microflora to resist colonization by an invading bacterium (45). As an example, when healthy human volunteers are fed *Escherichia coli* strains isolated from their own feces, those strains do not colonize (1). Due to colonization resistance, studies aimed at determining the nutritional basis of *E. coli* intestinal colonization are difficult, if not impossible, with conventional animals.

The nutritional basis of *E. coli* intestinal colonization can be studied in the streptomycin-treated mouse. Streptomycin treatment alters the microecology of the cecum, decreasing the populations of facultative anaerobes (enterococci, streptococci, and lactobacilli) and strict anaerobes (lactobacilli and bifidobacteria). Accompanying these changes in microflora is a general decrease in the concentration of volatile fatty acids, which may play a role in the natural resistance of the conventional mouse intestine to invading *E. coli* strains (18, 19). Nevertheless, populations of the genera *Bacteroides* and *Eubacterium* in cecal contents of streptomycin-treated mice remain largely unchanged (19). Moreover, the overall number of strict anaerobes in the cecal contents of streptomycin-treated and conventional mice are essentially identical (1×10^9 to 2×10^9 CFU/g of contents) (19). Therefore, while the streptomycin-

treated mouse model is not perfect, invading microorganisms must compete for nutrients with a large number of strict anaerobes in the intestine, just as they do in conventional animals.

By using the streptomycin-treated mouse model, it has been shown that when 10^5 CFU of either *E. coli*, *Salmonella enterica* serovar Typhimurium, or *Klebsiella pneumoniae* strains are fed to streptomycin-treated mice, they grow from low numbers at 5 h postfeeding (10^5 CFU/g of feces) to high numbers (10^8 to 10^9 CFU/g of feces) within 1 to 3 days postfeeding (11, 29, 33, 34). Following this initiation stage, a maintenance stage is reached in which stable populations of 10^6 to 10^7 CFU/g of feces persist indefinitely (11, 29, 33, 34). Studies of the human commensal *E. coli* F-18 and K-12 strains strongly implicated D-gluconate, which is catabolized via the Entner-Doudoroff (ED) pathway, as being important for colonization of the streptomycin-treated mouse large intestine during both initiation and maintenance (8, 35, 43, 44). Furthermore, it has been shown that the likely source of the gluconate is mouse intestinal tissue and not food (44).

Since *E. coli* colonization of the mouse intestine appears to require the ability to grow in mucus (23, 30, 34, 43, 44, 46), the power of DNA microarrays was used to focus attention on identifying genes induced by growth in mouse cecal mucus in vitro relative to growth in minimal medium containing glucose as the carbon source. This approach allowed the identification of additional nutrients, including *N*-acetyl-D-glucosamine, *N*-acetylneuraminic (sialic) acid, L-fucose, D-ribose, and D-glucuronate, as being necessary for the maximum ability of *E. coli* MG1655 to colonize the intestine (8). *E. coli* MG1655 was chosen as the strain to be tested since it has been completely sequenced (6).

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TABLE 1. Bacterial strains used in this study

E. coli strain[a]	Relevant characteristic(s)	Source or reference
MG1655 Str^r	Spontaneous streptomycin-resistant mutant of MG1655	29
MG1655 Str^r Nal^r (MG1655)	Spontaneous nalidixic acid-resistant mutant of MG1655 Str^r	29
MG1655 ΔgntK::cam ΔidnK (MG1655 ΔgntK ΔidnK)	Lacks gluconate kinases I and II and fails to grow using gluconate as sole carbon and energy source	This study
MG1655 Str^r Δedd::kan (MG1655 Δedd)	Lacks 6-phosphogluconate dehydratase and grows poorly using gluconate as sole carbon and energy source	8
MG1655* Str^r Nal^r (MG1655*)	500-bp deletion immediately downstream of ISI in regulatory region of flhD, extending into flhD	Colony isolated from feces 15 days postfeeding of MG1655 Str^r Nal^r; this study
MG1655 Str^r Δedd*::kan (MG1655 Δedd*)	2,384-bp deletion immediately downstream of ISI in regulatory region of flhD through flhD, flhC, and motA and into motB	Colony isolated from feces 20 days postfeeding of MG1655 Str^r Δedd::kan; this study
MG1655 Str^r ΔflhD::cam (MG1655 ΔflhD)	546-bp deletion encompassing region immediately downstream of ISI in regulatory region of flhD and into flhD	This study
MG1655 Str^r Δedd::kan ΔflhD::cam (MG1655 Δedd ΔflhD)	546-bp deletion encompassing region immediately downstream of ISI in regulatory region of flhD and into flhD	This study

[a] The designations in parentheses are those used in the text.

In the present study, while examining the role of the ED pathway in the ability of E. coli MG1655 to colonize the mouse intestine, we made the discovery that the mouse intestine selects nonmotile MG1655 flhDC mutants that are unable to make the master regulator of flagellar biosynthesis. We show that these mutants grow significantly faster than their parent on several sugars that have been shown previously to be involved in the colonization process, that they are better colonizers of the mouse intestine than their parent, and that the mutations in the flhDC operon are indeed responsible for these effects.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The bacterial strains used in this study are listed in Table 1. Luria broth (LB) was made as described by Revel (39). Luria agar is LB containing 12 g of Bacto Agar (Difco) per liter. MacConkey agar (Difco) was prepared according to package instructions. M9 minimal medium (27) was supplemented with reagent grade N-acetyl-D-glucosamine (0.2%, wt/wt), L-fucose (0.2%, wt/wt), D-glucose (0.2%, wt/wt), D-galactose (0.2%, wt/wt), D-gluconate (0.2%, wt/wt), D-glucuronate (0.2%, wt/wt), glycerol (0.4%, wt/wt), D-mannose (0.2%, wt/wt), D-ribose (0.2%, wt/wt), potassium acetate (0.4%, wt/wt), or sodium succinate (0.4%, wt/wt). Cultures (10 ml) were grown at 37°C with shaking in 125-ml tissue culture bottles. Inocula were prepared as follows. Overnight cultures on LB were started from a single colony on Luria agar plates. The LB cultures were washed twice in M9 minimal medium (no carbon source), and 10-μl volumes of the washed cultures were transferred to M9 minimal glucose medium and then incubated overnight. These cultures were washed twice as described above, and M9 minimal media (10 ml) containing various carbon sources were then inoculated with 10-μl volumes of the washed cultures, which were grown overnight. The next morning, each culture was diluted to an A_{600} of about 0.045 into fresh M9 medium (30 ml) containing the same carbon source and the cultures were incubated at 37°C with shaking in 125-ml tissue culture bottles. Growth was monitored spectrophotometrically (A_{600}) with a Pharmacia Biotech Ultrospec 2000 UV/Visible Spectrophotometer. Generation times were calculated during exponential phase from three independent experiments.

In vitro growth in mouse cecal mucus. Mouse cecal mucus was isolated as previously described (9). Briefly, mice (5 to 8 weeks old) were fed Charles River Valley Rat, Mouse, and Hamster Formula for 5 days after being received. The drinking water was then replaced with sterile distilled water containing streptomycin sulfate (5 g/liter). Twenty-four hours later, the mice were sacrificed by CO_2 asphyxiation and their ceca were removed. The cecal contents were collected for use in growth experiments (see below), and any remaining cecal contents were washed out with sterile distilled water. Cecal mucus was scraped into HEPES-Hanks buffer (pH 7.4), centrifuged, and sterilized by UV irradiation as described previously (9) and was adjusted to a concentration of 1 mg/ml with respect to

protein with sterile HEPES-Hanks buffer (pH 7.4) as described previously (9). Five-milliliter aliquots were inoculated at an A_{600} of about 0.1 with either MG1655, MG1655*, MG1655 Δedd, or MG1655 Δedd*. Three 1-ml aliquots of each strain in cecal mucus were transferred to polystyrene cuvettes, which were then incubated standing at 37°C in a water bath, and the A_{600} of each culture was determined hourly. Uninoculated sterile cecal mucus was used as a blank. Generation times were determined when growth was in exponential phase, as determined from semilogarithmic plots. Cecal contents (1-ml aliquots) were inoculated to about 10^4 CFU/ml with each of the strains as described above for cecal mucus, the cultures were incubated standing at 37°C, and samples taken at 0, 2, 4, 6, and 24 h were diluted, plated, and counted as described previously (30, 46).

Mouse colonization experiments. The method used to compare the large-intestine-colonizing abilities of E. coli strains in mice has been described previously (43, 44, 46). 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 resident facultative bacteria (28). Following 18 h of starvation for food and water, the mice were fed 1 ml of 20% (wt/vol) sucrose containing LB-grown MG1655 strains as described in Results. After ingestion of the bacterial suspension, both the food (Charles River Valley Rat, Mouse, and Hamster Formula) and streptomycin-water were returned to the mice and 1 g of feces was collected after 5 h, after 24 h, and on odd-numbered days at the indicated times. 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 purchase from Sigma-Aldrich (St. Louis, MO). All plates were incubated for 18 to 24 h at 37°C prior to counting. In some experiments, mice were precolonized for 9 days, starved overnight for food and streptomycin-water, and then fed 10^5 CFU of a second strain, after which food and streptomycin-water were returned. Each colonization experiment was performed at least twice with essentially identical results. Pooled data from at least two independent experiments are presented in the figures.

Motility. Motility agar is LB containing 3.5 g of Bacto Agar per liter. Colonies were toothpicked to motility agar, and plates were incubated at 37°C for 8 h and then overnight and at each time were examined for growth and spreading.

Serotyping. Serotyping of O and H antigens was performed with specific antisera produced by the World Health Organization International Escherichia and Klebsiella Centre, Statens Seruminstitut, Copenhagen, Denmark.

Mutant construction. Primers used to construct deletion mutants were designed according to the MG1655 genome database (6). DNA procedures were as described previously (30). The MG1655 ΔgntK ΔidnK double-deletion mutant (Table 1) was constructed by allelic exchange as described by Datsenko and Wanner (10). Initially, 455 bp were deleted from the idnK (gluconate kinase II) gene by using a PCR product containing the chloramphenicol resistance cassette (10) flanked upstream and downstream by idnK-specific DNA sequences. The chloramphenicol resistance cassette was then removed (10), leaving a deletion in idnK beginning 42 bp downstream of the ATG start codon and ending 62 bp

upstream of the TGA stop codon. The *idnK* deletion primers (uppercase letters, MG1655 DNA; lowercase letters, chloramphenicol resistance cassette DNA) were as follows: primer 1, 5′-ATGGCGGGTGAAAGCTTTATTTTGATG GGCGTTTCAGGGAGTGGTgtgtaggctggagctgcttcg-3′; primer 2, 5′-AGGCGC TGCCCTCTTTCGCACATATTCTGTTTTGTCGTATCGCCAcatatgaatatcctc cttagt-3′. The MG1655 Δ*idnK* mutant was then used to construct the MG1655 Δ*gntK* Δ*idnK* double-deletion mutant. Four hundred base pairs, beginning 42 bp downstream of the ATG start codon and ending 41 bp upstream of the TAA stop codon, were deleted from the *gntK* (gluconate kinase I) gene of the MG1655 Δ*idnK* mutant with PCR product containing the chloramphenicol resistance cassette flanked by upstream and downstream *gntK*-specific sequences as described by Datsenko and Wanner (10). The *gntK* deletion primers (uppercase letters, MG1655 DNA; lowercase letters, chloramphenicol resistance cassette DNA) were as follows: primer 1, 5′-ATGGGCGTATCGGGCAGCGGCAAA TCTGCGGTCGCCAGTGAAGTGcatatgaatatcctccttagt-3′; primer 2, 5′-CTT ATTTGCCTTTTTTAATAACCTCAATGGTGCTTGCCACAACACgtgtaggct ggagctgcttcg-3′. The MG1655 Δ*gntK* Δ*idnK* double-deletion mutant was confirmed phenotypically as being unable to grow in M9 minimal medium with gluconate as the sole carbon and energy source and by sequencing (see below).

An MG1655 Δ*flhD* deletion mutant and an MG1655 Δ*edd* Δ*flhD* double-deletion mutant (Table 1) were constructed by removing 546 bp originating immediately downstream of IS*1* in the *flhD* promoter and extending into *flhD* in MG1655 and in MG1655 Δ*edd* with a PCR product containing the chloramphenicol resistance cassette flanked upstream and downstream by *flhD*-specific sequences as described by Datsenko and Wanner (10). The specific PCR primers used to construct and confirm the *flhD* deletions (uppercase letters, MG1655 DNA; lowercase letters, chloramphenicol resistance cassette DNA) were as follows: primer 1 (immediately downstream of IS*1*), 5′-TTAAGTAATTGAGTG TTTTGTGTGATCTGCATCACGCATTATTGAAAATgtgtaggctgga gctgcttcg-3′; primer 2 (within *flhD*), 5′-AGGCCCTTTTCTTGCGCAGCGCTTCTTCAG GCTGATTAACATCATTCAGcatatgaatatcctccttagt-3′. The mutants were confirmed phenotypically by failure to spread on motility agar, genetically by PCR with primers specific to upstream and downstream flanking sequences, and by sequencing (see below).

The MG1655 Δ*edd* mutant strain was constructed previously (8). The primers upstream and downstream of the *edd* gene used to amplify both the 2,300-bp wild-type *edd* gene and the 1,900-bp Δ*edd* gene containing the kanamycin resistance cassette (8) were as follows: forward, 5′-GGCTAATTGCGAACTGTG CAC-3′; reverse, 5′-CGGTAACATGATCTTGCGCAGA-3′.

Determination of the sizes of the deletions in MG1655* and MG1655 Δ*edd**. The following primers used to define the size of the deletion in MG1655* were those described by Barker et al. (2): forward, 5′-CCTGTTTCATTTTTGCTT GCTAGC-3′; reverse (downstream of *flhD*), 5′GGAATGTTGCGCCTCACC G-3′. Those used for MG1655 Δ*edd** were as follows: forward, same as for MG1655*; reverse (within *cheA*), 5′-CGCTGAAGCCAAAAGTTCCTGC-3′.

Sequencing. DNA sequencing was done at the URI Genomics and Sequencing Center, University of Rhode Island, Kingston, with the CEQ8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA) The 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 *idnK* were as follows: primer 1 (upstream of *idnK*), 5′-CGCATAACGTGATGTGCCTTG-3′; primer 2 (downstream of *idnK*), 5′-GCCGATAAAGTGGTGAATAGC. Primer 2 was also used in the *idnK* sequencing reaction. The primers used to amplify PCR products for sequencing to determine the precise location of the deletion in *gntK* were as follows: primer 1 (upstream of *gntK*), 5′-ATTCGTGGCGAATCTGTGACAC-3′; primer 2 (downstream of *gntK*), 5′-TAAGATCTTGCCAAACATAGCTC-3′. Primer 2 was also used in the *gntK* sequencing reaction. The primers used to amplify PCR products for sequencing to determine the precise locations of the deletions in MG1655* and MG1655 Δ*edd** were identical to those used to define the sizes of the deletions. The primer used in the sequencing reactions was 5′-GGGAAA GCTGCACGTAATCAGC-3′.

Statistics. Where indicated, means derived from triplicate samples were compared by Student's *t* test (*P* values).

RESULTS

The ED pathway is important for *E. coli* MG1655 growth in the mouse intestine. The *edd* (ED dehydratase) gene, which is the promoter-proximal gene in the *edd-eda* operon, encodes 6-phosphogluconate dehydratase, which converts 6-phospho-

TABLE 2. Growth of MG1655, MG165*, MG1655 Δ*edd*, and MG1655 Δ*edd** on various carbon sources[a]

Carbon source	Mean generation time (min) ± SD (*n* = 3)			
	MG1655	MG1655 Δ*edd*	MG1655 Δ*edd**	MG1655*
Fucose	109 ± 4[abC]	115 ± 2[aDE]	101 ± 4[bDf]	98.8 ± 1[CEf]
Galactose	169 ± 6[aBc]	171 ± 6[aDe]	152 ± 2[BDf]	159 ± 5[cef]
Glucose	87 ± 6[abc]	93 ± 5[ade]	89 ± 6[bdf]	85 ± 4[cef]
N-AcGlcNH₂[b]	92 ± 1[ABC]	111 ± 6[ADE]	95 ± 1[BDF]	80 ± 5[CEF]
Gluconate	77 ± 5[ABc]	178 ± 4[ADE]	135 ± 1[BDF]	70 ± 4[cEF]
Glucuronate	93 ± 2[ABC]	103 ± 5[ADE]	85 ± 2[BDf]	80 ± 3[CEf]
Glycerol	114 ± 2[ABC]	124 ± 1[ADE]	103 ± 2[BDf]	93 ± 8[CEf]
Mannose	159 ± 6[ABC]	177 ± 1[ADE]	136 ± 2[BDf]	135 ± 3[CEf]
Ribose	134 ± 2[ABC]	144 ± 4[ADE]	125 ± 3[BDF]	113 ± 1[CEF]
Acetate	203 ± 14[ABC]	242 ± 6[ADE]	176 ± 6[BDf]	172 ± 12[CEf]
Succinate	106 ± 4[aBC]	107 ± 4[aDE]	87 ± 3[BDf]	88 ± 1[CEf]
Cecal mucus	105 ± 2[ABC]	117 ± 3[ADE]	100 ± 1[BDf]	90 ± 2[CEF]

[a] For each carbon source, the comparison of the generation time of any two strains is indicated by the same superscript letter, e.g., MG1655 and MG1655 Δ*edd* by the letter a, MG1655 Δ*edd* and MG1655 Δ*edd** by the letter d, MG1655 Δ*edd** and MG1555* by the letter f, etc. Lowercase letters indicate that the two generation times are not significantly different, whereas uppercase letters indicate that the two generation times are significantly different. See text for *P* values.
[b] N-AcGlcNH₂, *N*-acetylglucosamine.

gluconate to 2-keto-3-deoxy-6-phosphogluconate in the ED pathway, the primary route for gluconate catabolism. The *eda* (ED aldolase) gene encodes 2-keto-3-deoxy-6-phosphogluconate aldolase, which converts 2-keto-3-deoxy-6-phosphogluconate to glyceraldehye-3-phosphate and pyruvate. Gluconate can also be catabolized secondarily via the pentose phosphate pathway. The MG1655 Δ*edd* mutant therefore grows on gluconate as a sole carbon and energy source with a generation time of about 180 min, whereas wild-type MG1655 grows with a generation time of about 80 min. Expression of *eda* is not only required for the maximum rate of gluconate catabolism but is absolutely required for growth of *E. coli* on glucuronate as a sole carbon and energy source (4).

The MG1655 Δ*edd* mutant, which was previously shown to be a poor colonizer of the mouse intestine, has been described previously (8). It has a kanamycin resistance cassette (10) in place of the *edd* gene; however, the kanamycin resistance cassette insertion has a minimal, if any, effect on the downstream expression of *eda* since the MG1655 Δ*edd* mutant grows well with glucuronate as a sole carbon and energy source (Table 2). Since the MG1655 Δ*edd* mutant still grows slowly on gluconate, we constructed MG1655 Δ*gntK* Δ*idnK* (Table 1), which is unable to use gluconate as a carbon and energy source, to assess the true impact of gluconate catabolism on MG1655 intestinal colonization (4). MG1655 Δ*gntK* Δ*idnK* fails to grow on gluconate as a carbon source because it contains neither gluconate kinase I (GntK) nor gluconate kinase II (IdnK) and therefore cannot make 6-phosphogluconate from gluconate. As shown previously (8), MG1655 Δ*edd* has a major colonization defect in the presence of wild-type MG1655 characterized by a failure to grow rapidly during the initial 24 h (the initiation stage), a significant drop between days 1 and 3 postfeeding (*P* < 0.001, Student's *t* test), and a subsequent slow but continuous reduction thereafter (the maintenance stage) such that by 15 days postfeeding it colonized at a level about 3.5 orders of magnitude lower than that of MG1655 (Fig. 1A). In contrast, the MG1655 Δ*gntK* Δ*idnK* double-deletion mutant had a defect in initiation, but by day 15 postfeeding it colonized at a
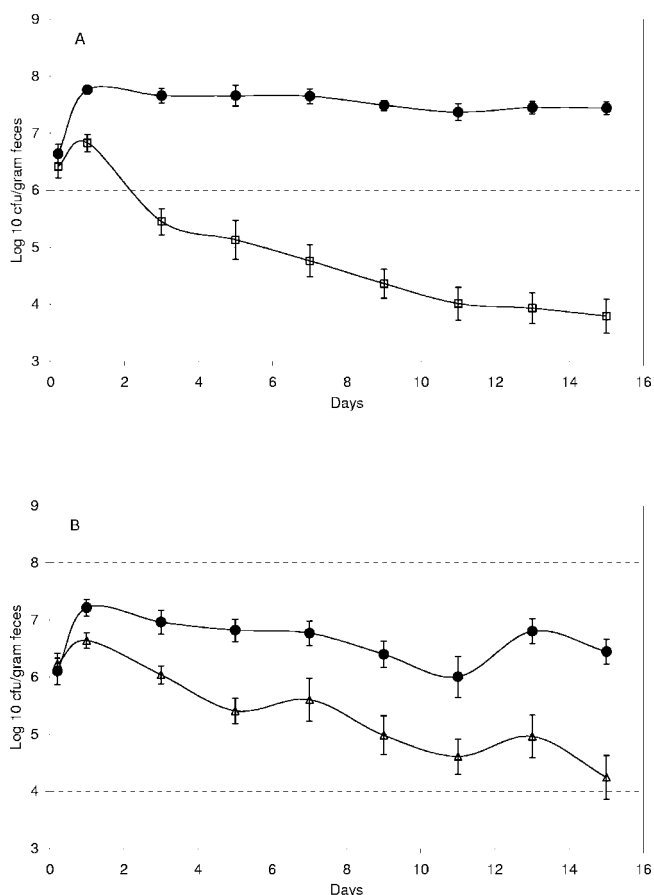
FIG. 1. *E. coli* MG1655 Δ*gntK* Δ*idnK* and MG1655 Δ*edd* coloniza-tion of the mouse large intestine. Sets of three mice were fed either 10^5 CFU of *E. coli* MG1655 Str^r Nal^r (●) and 10^5 CFU of *E. coli* MG1655 Str^r Δ*edd*::*kan* (□) (A) or 10^5 CFU of *E. coli* MG1655 Str^r Nal^r (●) and 10^5 CFU of *E. coli* MG1655 Str^r Δ*gntK*::*cam* Δ*idnK* (△) (B). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars represent the standard error of the log$_{10}$ mean number of CFU per gram of feces. The data presented are from six experiments (18 mice) with MG1655 Δ*edd* and four experiments (12 mice) with MG1655 Δ*gntK* Δ*idnK*.

FIG. 2. Growth of low numbers (10^5 CFU/mouse) of *E. coli* MG1655 bacteria in the presence of high numbers (10^{10} CFU/mouse) of *E. coli* MG1655 Δ*edd* bacteria in the mouse large intestine. (A) Sets of three mice were fed 10^5 CFU of *E. coli* MG1655 Str^r Nal^r (●) and 10^{10} CFU of *E. coli* MG1655 Str^r Δ*edd*::*kan* (□). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars representing the standard error of the log$_{10}$ mean number of CFU per gram of feces for each set of six mice are presented for each time point. (B) Sets of three mice were fed 10^5 CFU of *E. coli* MG1655 Str^r Δ*edd*::*kan* (□) and, on day 10 postfeeding, 10^5 CFU of *E. coli* MG1655 Str^r Nal^r (●). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars representing the standard error of the log$_{10}$ mean number of CFU per gram of feces for six mice are presented for each time point.

level only about 2.0 orders of magnitude lower than that of MG1655 (Fig. 1B). These results suggested the possibility that while gluconate is a major carbon source for MG1655 in the intestine, an intact ED pathway might also be used for catabolism of other carbon sources that are important for colonization. Indeed, MG1655 grows faster than the MG1655 Δ*edd* strain on a variety of carbon sources that are not directly catabolized via the ED pathway, as described immediately below.

Growth of MG1655 and MG1655 Δ*edd* on various sole carbon and energy sources. The generation times of MG1655 and MG1655 Δ*edd* on a variety of carbon sources were determined as described in Materials and Methods. As shown in Table 2, although MG1655 Δ*edd* grew at about the same rate as MG1655 on glucose ($P > 0.10$), fucose ($P > 0.10$), and succi-nate ($P > 0.10$), it grew 10 to 20% more slowly than MG1655 on *N*-acetylglucosamine ($P < 0.01$), glucuronate ($P < 0.05$), glycerol ($P < 0.002$), mannose ($P < 0.01$), ribose ($P < 0.02$), and acetate ($P < 0.02$). Therefore, a functional ED pathway is
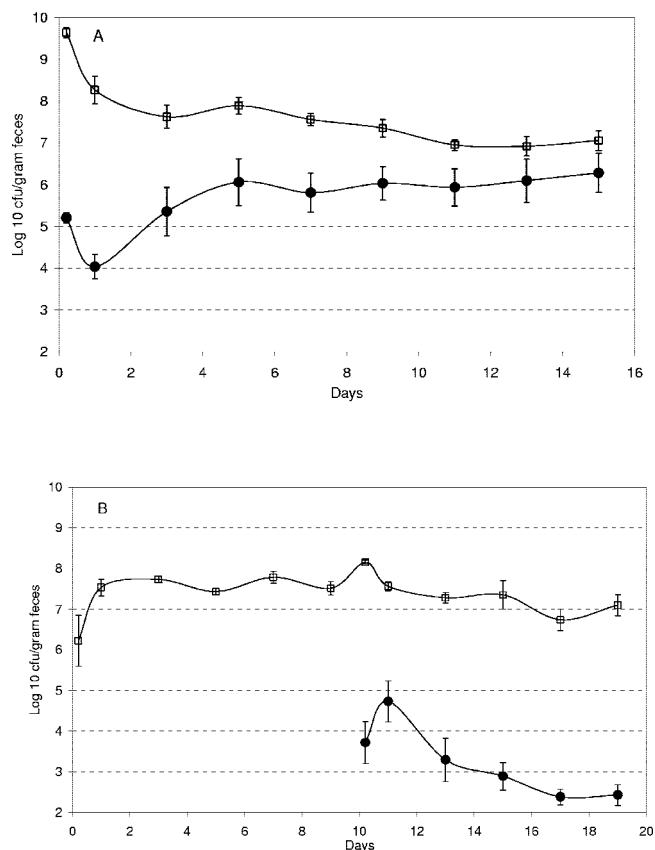
essential for maximum growth rates of MG1655 on a variety of different carbon sources, including several that have been shown to be utilized by MG1655 during colonization (8). By contrast, with the exception of gluconate, MG1655 and the MG1655 Δ*gntK* Δ*idnK* double-deletion mutant grew at the same rate on glucose, fucose, succinate, *N*-acetylglucosamine, glucuronate, glycerol, ribose, and acetate (data not shown).

Intestinal growth of low numbers of wild-type MG1655 bacteria in the presence of high numbers of the MG1655 Δ*edd* mutant. Mice were fed high numbers (10^{10} CFU/mouse) of bacteria of the MG1655 Δ*edd* mutant and low numbers (10^5 CFU/mouse) of bacteria of the MG1655 wild-type strain. Over a period of several days, the level of the wild-type strain sig-nificantly increased in the intestine and approached the level of the MG1655 Δ*edd* mutant (Fig. 2A), which was not surprising since the wild-type strain utilizes several carbon sources better than MG1655 Δ*edd* does (Table 2). In a control experiment,
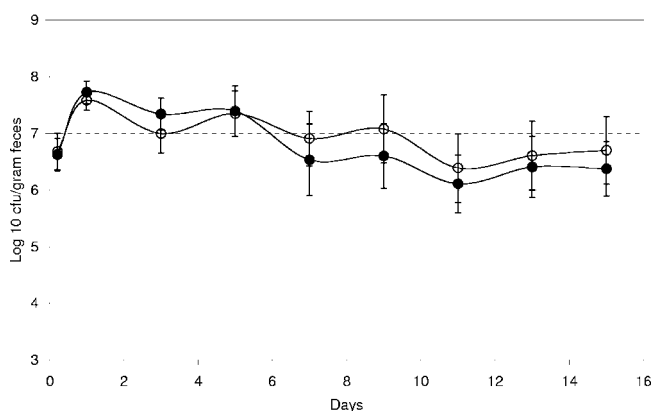
FIG. 3. *E. coli* MG1655 and MG1655 Δ*edd** colonization of the mouse large intestine. Sets of three mice were fed 10^5 CFU of *E. coli* MG1655 Str^r Nal^r (●) and 10^5 CFU of *E. coli* MG1655 Str^r Δ*edd**::*kan* (○). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars representing the standard error of the $\log_{10}$ mean number of CFU per gram of feces for six mice are presented for each time point.

when mice were fed high numbers (10^{10} CFU/mouse) of bacteria of the wild-type MG1655 strain (resistant to streptomycin) and low numbers (10^5 CFU/mouse) of bacteria of the same wild-type strain (resistant to streptomycin and nalidixic acid), the bacteria maintained the initial ratio of their input values, as expected of two strains that use all nutrients equally well (data not shown).

To further explore the ability of the wild-type MG1655 strain to outcompete the MG1655 Δ*edd* mutant, mice were precolonized with the MG1655 Δ*edd* mutant for 10 days and then fed low numbers of bacteria of the MG1655 wild-type strain (10^5 CFU/mouse). Surprisingly, the wild-type MG1655 strain failed to grow to the level of the MG1655 Δ*edd* mutant in the intestine and, in fact, stabilized at only about 10^2 CFU/g of feces (Fig. 2B). These data suggested that the MG1655 Δ*edd* mutant either adapted physiologically in the intestine such that it could compete well with the nonadapted MG1655 wild type or that the intestine selected a mutant of the MG1655 Δ*edd* strain that was a better colonizer than the input strain. In order to determine which of these hypotheses was correct, one colony of MG1655 Δ*edd* present in feces at 20 days postfeeding was designated MG1655 Δ*edd** and selected for further study.

MG1655 Δ*edd** is a better mouse large intestine colonizer than MG1655 Δ*edd*. After confirmation by PCR that the *edd* deletion in MG1655 Δ*edd** was intact (see Materials and Methods), MG1655 Δ*edd** and wild-type MG1655 bacteria were fed together to mice in low numbers (10^5 CFU/mouse). Under these conditions, both the wild-type MG1655 strain and MG1655 Δ*edd** cocolonized at a level between 10^6 and 10^7 CFU/g of feces (Fig. 3). Therefore, MG1655 Δ*edd** appeared to be a genetically stable derivative of the original MG1655 Δ*edd* strain that was able to colonize as well as wild-type MG1655.

Growth of MG1655 Δ*edd** on various carbon sources. Since MG1655 Δ*edd** was found to be a better colonizer of the mouse large intestine than MG1655 Δ*edd*, it was of interest to determine the in vitro growth rates of the two strains on various carbon sources. As shown in Table 2, MG1655 Δ*edd** and MG1655 Δ*edd* grew equally well on glucose as a sole carbon

and energy source ($P > 0.10$). In contrast, MG1655 Δ*edd** grew 15 to 30% faster than MG1655 Δ*edd* on acetate ($P < 0.001$), fucose ($P < 0.01$), galactose $P < 0.01$), *N*-acetylglucosamine ($P < 0.02$), gluconate ($P < 0.001$), glucuronate ($P < 0.01$), glycerol ($P < 0.001$), mannose ($P < 0.001$), ribose ($P < 0.01$), and succinate ($P < 0.002$). It should be noted that although MG1655 Δ*edd** grew faster than MG1655 Δ*edd* with gluconate as the sole carbon source ($P < 0.001$), it still grew far more slowly on gluconate ($P < 0.001$) than wild-type MG1655 (Table 2). However, MG1655 Δ*edd** grew about 10 to 20% faster than wild-type MG1655 on glucuronate ($P < 0.02$), glycerol ($P < 0.002$), mannose ($P < 0.02$), ribose ($P < 0.02$), and succinate ($P < 0.01$) (Table 2). These data suggest that the improved colonizing ability of MG1655 Δ*edd** relative to MG1655 may be due to its ability to grow more rapidly than wild-type MG1655 on a number of carbon sources present in the intestine.

Low numbers of wild-type MG1655 bacteria cannot grow to high numbers in the presence of high numbers of MG1655 Δ*edd** mutant bacteria. The results described above indicated that wild-type MG1655 was able to use gluconate better, but grew more slowly on several other carbon sources, compared to MG1655 Δ*edd** (Table 2). Since bacteria of both strains were equally good colonizers when they were simultaneously fed to mice in low numbers (Fig. 3), it became of interest to determine whether low numbers of bacteria of wild-type strain MG1655 could grow in the intestine in the presence of high numbers of bacteria of the MG1655 Δ*edd** strain, i.e., whether the gluconate concentration was high enough during initiation to overcome the advantage of MG1655Δ*edd** on other sugars. When mice were fed 10^{10} CFU of the MG1655 Δ*edd** strain and 10^5 CFU of the wild-type MG1655 strain, the wild type was unable to colonize in the presence of high numbers of MG1655 Δ*edd** bacteria and was, in fact, rapidly eliminated (Fig. 4A). This result can be compared to that described above (Fig. 2A), where low numbers of bacteria of the wild-type MG1655 strain were able to grow to much higher numbers in the intestine in the presence of high numbers of MG1655 Δ*edd* bacteria. These data suggest that the more efficient use of carbon sources by MG1655 Δ*edd** prevented wild-type MG1655 from growing despite its advantage in the utilization of gluconate. Thus, it was of interest to test the hypothesis that the concentration of gluconate in the mouse intestine is not high enough to confer a growth advantage on wild-type MG1655 over the MG1655 Δ*edd** strain. In support of this view, when 2% gluconate was added to the drinking water, low numbers of wild-type MG1655 bacteria (10^5 CFU/mouse) were able to grow to much higher numbers in the presence of high numbers of MG1655 Δ*edd** bacteria (10^{10} CFU/mouse) (Fig. 4B).

In further support of the view that increased gluconate availability could confer a growth advantage on the wild-type MG1655 strain able to use it, mice were precolonized with MG1655 Δ*edd** and at day 10 postfeeding were fed wild-type MG1655 (10^5 CFU/mouse) with (Fig. 5A) or without (Fig. 5B) 2% gluconate in the drinking water. Under these conditions, low numbers of wild-type MG1655 bacteria failed to grow to high numbers in the intestine in the presence of high numbers of precolonized MG1655 Δ*edd** bacteria unless gluconate was present in the drinking water (compare Fig. 5A and B). These data indicate that if gluconate were not
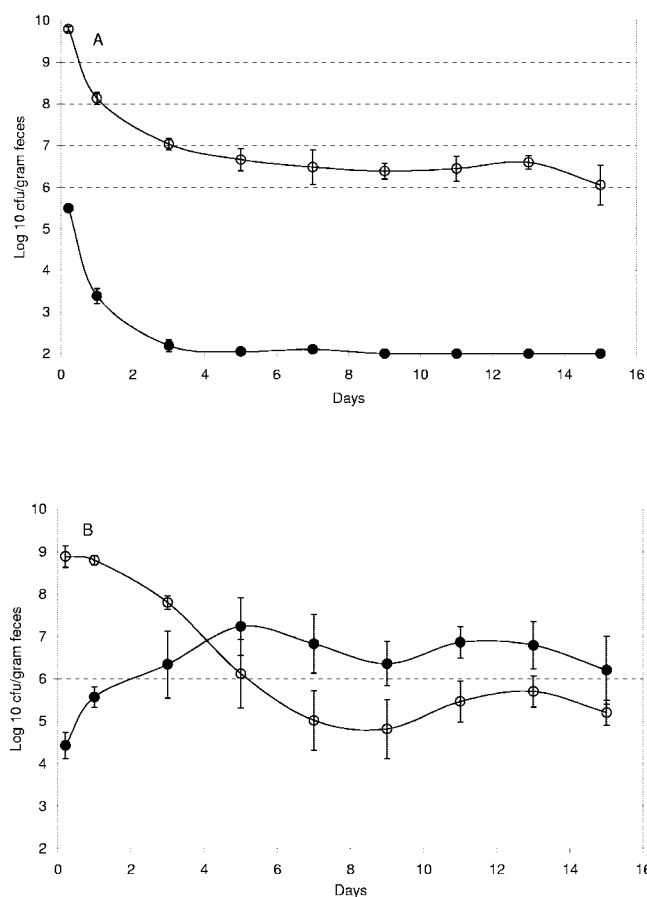
FIG. 4. Growth of low numbers (10^5 CFU/mouse) of *E. coli* MG1655 bacteria in the presence of high numbers (10^{10} CFU/mouse) of *E. coli* MG1655 Δ*edd** bacteria in the mouse large intestine. (A) Sets of three mice were fed 10^5 CFU of *E. coli* MG1655 Str^r Nal^r (●) and 10^{10} CFU of *E. coli* MG1655 Str^r Δ*edd**::*kan* (○). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars representing the standard error of the $\log_{10}$ mean number of CFU per gram of feces for each set of six mice are presented for each time point. (B) Same as panel A, except that the drinking water contained 2% gluconate.

FIG. 5. Growth of low numbers (10^5 CFU/mouse) of *E. coli* MG1655 bacteria in mice precolonized with *E. coli* MG1655 Δ*edd**. (A) Sets of three mice were precolonized for 10 days with *E. coli* MG1655 Str^r Δ*edd**::*kan* (○). On day10, the mice were fed 10^5 CFU of *E. coli* MG1655 Str^r Nal^r (●). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars representing the standard error of the $\log_{10}$ mean number of CFU per gram of feces for six mice are presented for each time point. (B) Same as in panel A, except that the drinking water contained 2% gluconate.

limiting at 10 days postfeeding, small numbers of MG1655 bacteria could still grow to much higher numbers in the intestine in the presence of high numbers of precolonized MG1655 Δ*edd** bacteria.

Isolation and characterization of MG1655*. To this point, the data suggested that MG1655 Δ*edd* utilizes not only gluconate poorly relative to MG1655 but a number of other carbon sources as well. In addition, at some point after mice were fed MG1655 Δ*edd*, it appeared that the mouse intestine selected better-colonizing mutants, among them MG1655 Δ*edd**, that were better able to grow on at least some of the sugars known to be present in mouse cecal mucus and utilized for growth in the intestine, i.e., fucose, gluconate, *N*-acetylglucosamine, glucuronate, mannose, and ribose (8, 13). It was therefore of interest to determine whether the mouse intestine would also select a mutant of the original wild-type MG1655 strain that was a better colonizer and grew faster than the original MG1655 strain on a variety of carbon sources. To that
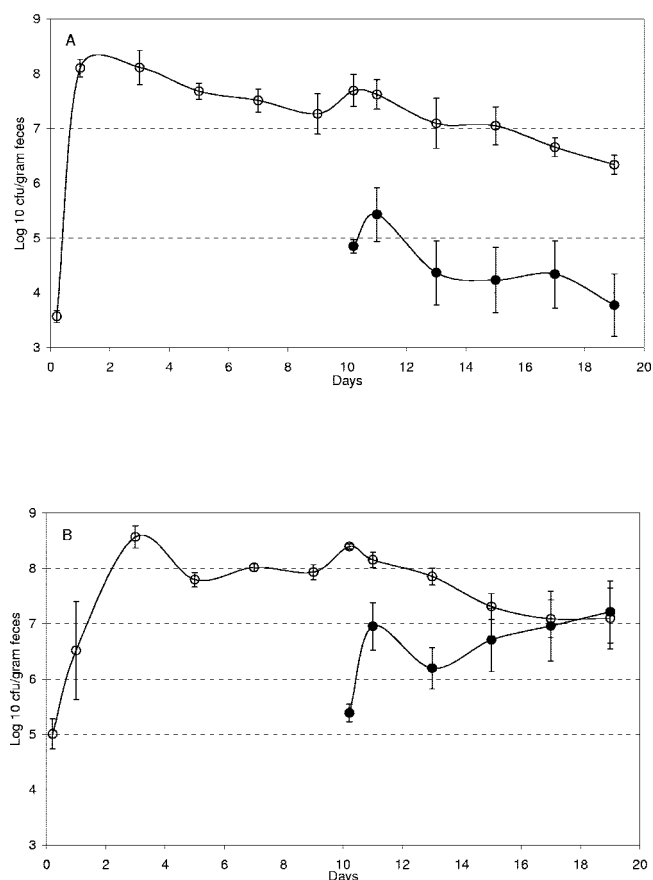
end, an MG1655 colony present in feces at 15 days postfeeding was selected for further testing and was designated MG1655*.

E. coli MG1655* grew at 10 to 25% faster rates than MG1655 on a variety of carbon sources, including acetate ($P < 0.01$), fucose ($P < 0.02$), *N*-acetylglucosamine ($P < 0.02$), glucuronate ($P < 0.01$), glycerol ($P < 0.01$), mannose ($P < 0.01$), ribose ($P < 0.001$), and succinate ($P < 0.01$), but not glucose ($P > 0.10$) (Table 2). It should also be noted that although wild-type MG1655 grew significantly slower than MG1655 Δ*edd** on several carbon sources, MG1655* and MG1655 Δ*edd** grew at about the same rate on acetate ($P > 0.10$), fucose ($P > 0.10$), glucuronate ($P = 0.05$), glycerol ($P > 0.05$), mannose ($P > 0.10$), and succinate ($P > 0.10$). In addition, MG1655* not only grew at about twice the rate of MG1655 Δ*edd** on gluconate ($P < 0.001$), it also grew about 10 to 15% faster than MG1655 Δ*edd** on *N*-acetylglucosamine ($P < 0.01$) and ribose ($P < 0.002$) (Table 2).

When MG1655* and MG1655 Δ*edd** bacteria were both fed to mice in low numbers (10^5 CFU/mouse), MG1655* proved to be a better colonizer than MG1655 Δ*edd** (Fig. 6A). Further-
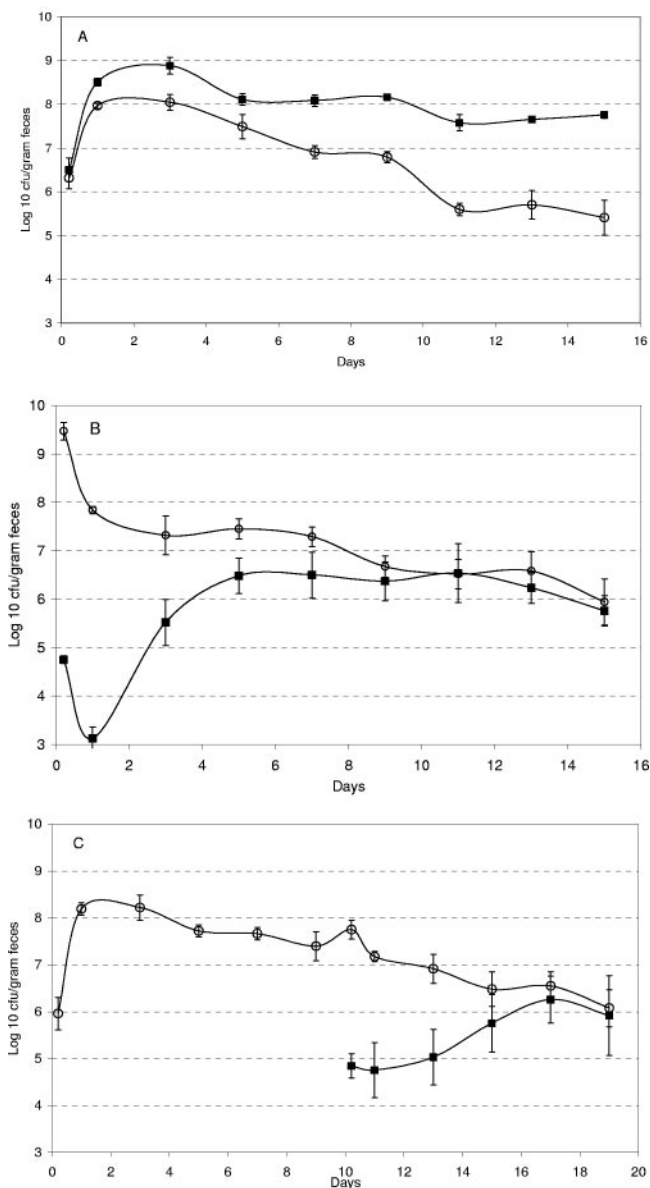
FIG. 6. Colonization of mice with *E. coli* MG1655* and *E. coli* MG1655 Δ*edd**. (A) Sets of three mice were fed 10^5 CFU of *E. coli* MG1655* Str^r Nal^r (■) and 10^5 CFU of *E. coli* MG1655 Str^r Δ*edd**::*kan* (○). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars representing the standard error of the log_{10} mean number of CFU per gram of feces for six mice are presented for each time point. (B) Same as panel A, except that sets of three mice were fed 10^5 CFU of *E. coli* MG1655* Str^r Nal^r (■) and 10^{10} CFU of *E. coli* MG1655 Str^r Δ*edd**::*kan* (○). (C) Same as panel A, except that mice were precolonized with *E. coli* MG1655 Str^r Δ*edd**::*kan* (○) and at day 10 were fed 10^5 CFU of *E. coli* MG1655* Str^r Nal^r (■).

more, unlike MG1655, low numbers of MG1655* bacteria (10^5 CFU/mouse) were able to grow to higher numbers in the intestines of mice simultaneously fed high numbers (10^{10} CFU/mouse) of MG1655 Δ*edd** bacteria (Fig. 6B). This was also the case when the mice were precolonized with MG1655 Δ*edd** (Fig. 6C). Therefore, the mouse intestine did indeed select an MG1655 mutant better able to utilize several carbon sources

and better able to colonize the mouse large intestine than its parent.

Growth of MG1655* and MG1655 Δ*edd in cecal mucus and cecal contents in vitro.** *E. coli* strains grow extremely well (generation times of 25 to 35 min, viable counts of about 10^9 CFU/ml) in cecal mucus (23, 30, 34, 43, 44, 46). To determine whether the MG1655* and MG1655 Δ*edd** strains had a growth advantage in cecal mucus, MG1655, MG1655*, MG1655 Δ*edd*, and MG1655 Δ*edd** were each inoculated separately into cecal mucus that had been diluted 50-fold into HEPES-Hanks buffer, pH 7.4 (1 mg/ml with respect to protein), at an A_{600} of 0.1. A_{600} readings were taken at hourly intervals for 8 h. All four strains grew to a final A_{600} of about 0.8 (approximately 1×10^8 to 2×10^8 CFU/ml), but MG1655* ($P < 0.001$) and MG1655 Δ*edd** ($P < 0.02$) grew more rapidly than their respective parents and MG1655* grew more rapidly than MG1655 Δ*edd** ($P < 0.001$) (Table 2). Moreover, MG1655 grew more rapidly in mucus than MG1655 Δ*edd* ($P < 0.02$) and MG1655 Δ*edd** grew more rapidly than MG1655 ($P < 0.01$) (Table 2). Thus, the ability of these strains to grow in cecal mucus in vitro correlated with their relative intestinal colonizing abilities.

While *E. coli* strains grow extremely well in cecal mucus in vitro, they fail to grow or grow poorly in cecal contents (feces) (23, 30, 34, 43, 44, 46), suggesting that colonization of the mouse intestine is due to the utilization of nutrients present in mucus for growth. However, strains of *E. coli* MG1655 isolated from feces during the maintenance stage of colonization had never been tested for the ability to grow in cecal contents in vitro. To that end, MG1655, MG1655*, MG1655 Δ*edd*, and MG1655 Δ*edd** were each inoculated into cecal contents isolated directly from the mouse cecum (10^4 CFU/ml). The cultures were incubated at 37°C, and viable counts were determined at 0, 2, 4, 6, and 24 h. Each strain doubled only twice in 6 h to about 4×10^4 CFU/ml and then remained at about the 6-h level at 24 h (data not shown). Therefore, neither MG1655* nor MG1655 Δ*edd** appears to be a better colonizer because it grows or survives better in cecal contents than its parent.

MG1655* and MG1655 Δ*edd are nonmotile.** MG1655, MG1655*, MG1655 Δ*edd*, and MG1655 Δ*edd** were subjected to 36 of the 41 biochemical tests listed in Table 5.3 of the 1984 edition of *Bergey's Manual of Systematic Bacteriology* (7) as described previously (30). The four strains were found to be identical with respect to all biochemical characteristics. Furthermore, the four strains contained approximately equal amounts of type 1 fimbriae. However, in contrast to MG1655 and MG1655 Δ*edd*, MG1655* and MG1655 Δ*edd** were nonmotile; i.e., they failed to tumble or swim after growth in LB and failed to spread on Luria motility agar (Fig. 7). The serotype of MG1655 and MG1655 Δ*edd* strains was OR:H48; i.e., both were rough and both contained the H48 flagellar antigen typical of K-12 strains. However, while both MG1655* and MG1655 Δ*edd** also typed as OR, neither strain contained the H48 flagellar antigen. Furthermore, in contrast to their parents, MG1655* and MG1655 Δ*edd** had no flagella when viewed by electron microscopy (data not shown). Therefore, MG1655* and MG1655 Δ*edd** are alike not only with respect to their more efficient utilization of carbon sources but in the loss of flagella and, consequently, motility.
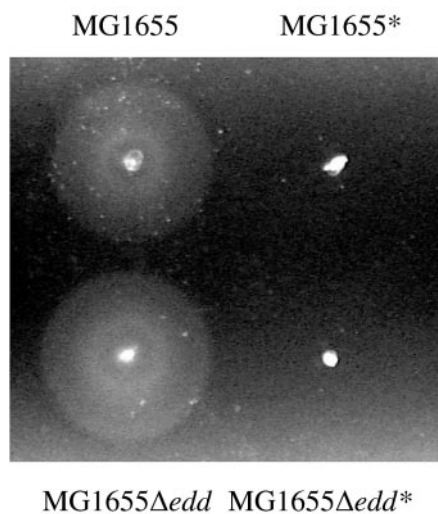
FIG. 7. Spread of *E. coli* MG1655 Str^r Nal^r, *E. coli* MG1655* Str^r Nal^r, *E. coli* MG1655 Str^r Δedd::*kan*, and *E. coli* MG1655 Str^r Δedd*::*kan* on Luria motility agar after 8 h at 37°C.

Although unlikely, it was possible that our MG1655 and MG1655 Δedd frozen stock cultures contained a high percentage of nonmotile mutants. To test this possibility, overnight LB cultures of MG1655 and MG1655 Δedd were plated on MacConkey agar and 600 individual colonies of each strain were tested for motility on motility agar. All colonies tested were motile, indicating that the strains being fed to the mice were predominantly motile and therefore that the nonmotile MG1655* and MG1655 Δedd* strains were selected in the mouse intestine following colonization.

Identification of MG1655* and MG1655 Δedd* genetic defects. *E. coli flhD* mutants have been reported to grow more rapidly than their parents in a tryptone-based medium (38). The *flhDC* operon, consisting of the *flhD* and *flhC* genes, encodes the master regulator of the 40-gene flagellar regulon (3), which has been reported to simultaneously regulate *E. coli* genes involved in galactose transport, the ED pathway, and the tricarboxylic acid cycle (36, 37). Additionally, in MG1655, an IS*1* element previously shown to be present in the regulatory region of the *flhDC* operon has recently been reported to enhance motility (2). It therefore seemed reasonable that MG1655* and MG1655 Δedd* might have been generated by IS*1*-mediated events, i.e., IS*1* deletion from the regulatory region of the *flhDC* operon or IS*1*-mediated deletion of adjacent *flhD*/*flhC* DNA. Indeed, PCR revealed that MG1655* had a 400- to 500-bp deletion in the *flhD*/*flhC* region and that MG1655 Δedd* had an about 2-kb deletion in the same region, thereby explaining why the strains are nonmotile (Fig. 8). Sequencing revealed that both MG1655* and MG1655 Δedd* retained IS*1* in the regulatory region of the *flhDC* operon but that the deletion in MG1655* (500 bp) had occurred immediately downstream of IS*1* and extended into *flhD*, whereas in MG1655 Δedd*, the deletion (2,384 bp) extended from immediately downstream of IS*1* through *flhD*, *flhC*, and *motA* and into *motB* (Fig. 8). The *motA* and *motB* genes encode proteins involved in flagellar motor rotation (5). Since the *flhDC* operon promoter was deleted in MG1655*, the *flhC* gene was

also presumably inactivated. The deleted genes in MG1655* and MG1655 Δedd* failed to be amplified by PCR (data not shown), showing that they were indeed lost rather than inserted elsewhere in their respective chromosomes.

Construction and characterization of $\Delta flhD$ mutants. To be sure that MG1655* and MG1655 Δedd* are better intestinal colonizers and utilize carbon sources better because of the defects in the *flhDC* operon, an MG1655 $\Delta flhD$ mutant and an MG1655 Δedd $\Delta flhD$ mutant were constructed (see Materials and Methods). The 546-bp deletion in both strains was designed to begin immediately downstream of the IS*1* element, i.e., to include the *flhDC* operon promoter and extend into *flhD*, thereby inactivating the entire operon (Fig. 8). Both strains were tested for colonizing ability relative to their parents and for utilization of ribose and mannose. By day 1 postfeeding, the MG1655 $\Delta flhD$ mutant had grown to a level about sixfold higher than the wild type in the intestine and beyond day 5 postfeeding maintained an about 20-fold advantage throughout the rest of the experiment (data not shown). Maintenance of the 20-fold advantage rather than a constantly increasing advantage would be expected if the intestine selected nonmotile, better-colonizing MG1655 mutants. Indeed, at 11 days postfeeding, of 600 MG1655 colonies tested for motility (100 from each of 6 mice), only 2 were found to be motile (1 in each of two mice). Similarly, the MG1655 Δedd $\Delta flhD$ mutant grew to a level about 60-fold higher than MG1655 Δedd by day1 postfeeding and colonized at a level of greater than 100-fold higher than MG1655 Δedd thereafter (data not shown). We were unable to determine the exact level of MG1655 Δedd at later times since MG1655 Δedd $\Delta flhD$ is resistant to both kanamycin and chloramphenicol, MG1655 Δedd is only resistant to kanamycin, and of 100 colonies toothpicked from kanamycin plates to chloramphenicol plates at each time point, none were sensitive to chloramphenicol. In addition, both the MG1655 $\Delta flhD$ and MG1655 Δedd $\Delta flhD$ mutants utilized both mannose and ribose at faster rates than their parents (data not shown). Therefore, the *flhDC* operon deletion mutants constructed in the laboratory behaved identically to those selected by the intestine, suggesting that loss of the *flhDC* operon is indeed responsible for improved utilization of carbon sources and better mouse intestine-colonizing ability.

DISCUSSION

The findings reported here can be considered in light of Freter's nutrient/niche theory, which postulates that the approximately 500 species indigenous to the mammalian gut (32) can coexist as long as each member of the microflora is able to utilize one or a few limiting nutrients better than all the others and that its rate of growth during the colonization process is at least equal to the washout rate from the intestine (14, 15, 16). According to the theory, the growth rate of a particular bacterium in the intestine is determined by the nature of the limiting nutrients it utilizes and the density to which it grows is determined by the available concentration of those nutrients. It is also possible for a species that does not compete well for limiting nutrients to colonize if it is able to adhere to the intestinal wall and thereby avoid washout (16). The available evidence suggests that *E. coli* MG1655 does not adhere to epithelial cells in the intestine but is limited to the mucus layer
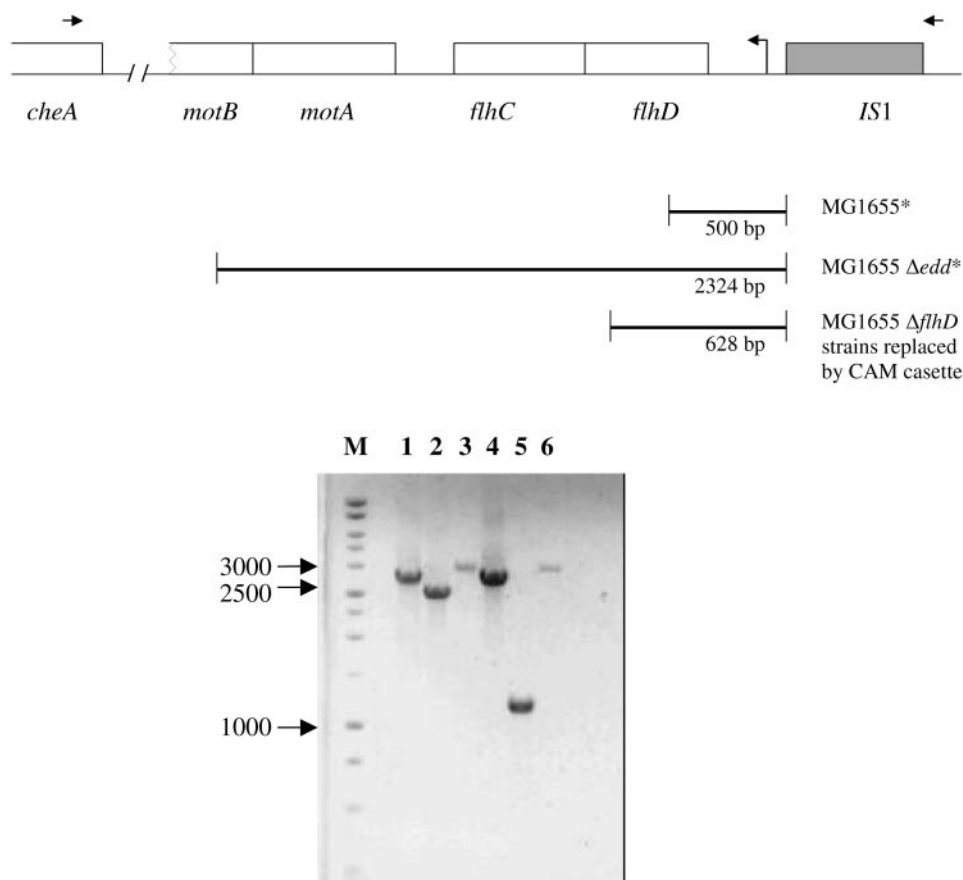
FIG. 8. Deletions in the *flhDC* region of MG1655*, MG1655 Δ*edd**, MG1655 Δ*flhD*, and MG1655 Δ*edd* Δ*flhD*. (Top) Bars indicate the positions of deletions in MG1655* (500 bp) and MG1655 Δ*edd** (2,324 bp) and a region (628 bp) that is deleted and replaced by the chloramphenicol resistance (CAM) cassette (1,154 bp) in MG1655 Δ*flhD* and MG1655 Δ*edd* Δ*flhD*. Small arrows indicate the positions of primers PCR2 (5′-CCTGTTTCATTTTTGCTTGCTAGC-3′) and cheAr (5′CGCTGAAGCCAAAAGTTCCTGC-3′). Bent arrow indicates the transcription start site. (Bottom) PCR products obtained with primers PCR2 and cheAr with wild-type MG1655 (lane 1), MG1655* (lane 2), MG1655 Δ*flhD* (lane 3), MG1655 Δ*edd* (lane 4), MG1655 Δ*edd** (lane 5), and MG1655 Δ*edd* Δ*flhD* (lane 6). Molecular size standards (lane M) are noted to the left (sizes are in base pairs).

and the luminal contents (29, 30), both of which turn over. While commensal strains of *E. coli* are present in both mucus and luminal contents, a large body of experimental evidence shows that growth is rapid in intestinal mucus both in vitro and in vivo but is either poor or completely inhibited in luminal contents (23, 30, 34, 43, 44, 46). It is therefore highly likely that the ability of a commensal *E. coli* strain to grow and survive in intestinal mucus plays a critical role in its ability to colonize the intestine. In support of this view, the better-colonizing strains selected by the mouse intestine, MG1655* and MG1655 Δ*edd**, grew more rapidly than their parents in cecal mucus in vitro (Table 2).

It had been previously shown that MG1655 utilizes gluconate, *N*-acetylglucosamine, and sialic acid as carbon sources for growth in the mouse intestine during the initiation stage of colonization and gluconate, glucuronate, mannose, fucose, and ribose for growth during the maintenance stage (8). The data presented here support the notion that it is the ability of MG1655* and MG1655 Δ*edd** to utilize several carbon sources better than their parents (Table 2) that makes them better colonizers of the mouse intestine. This finding has broad im-

plications with respect to colonization resistance. For example, low numbers of MG1655 bacteria were eliminated by high numbers of MG1655 Δ*edd** bacteria when both were fed simultaneously to mice (Fig. 4A) and failed to grow to high numbers in mice precolonized with MG1655 Δ*edd** (Fig. 5A). Therefore, selection of a mutant derivative of MG1655 Δ*edd* (MG1655 Δ*edd**) which uses several carbon sources better allowed the mouse colonized with this strain to resist colonization by MG1655.

The role of diet in microfloral stability is not clear (32). However, in the present study, we have shown that diet may play a role in minimizing colonization resistance as long as a specific preferred nutrient is not completely absorbed in the small intestine. That is, although it has been shown that the source of gluconate for *E. coli* colonization is the mouse intestinal tissue and not mouse chow (44), increasing the gluconate concentration in the intestine was possible since as much as 70% of the gluconate fed to animals reaches the cecum (20). Under these conditions, with 20 g/liter gluconate in the drinking water, small numbers of MG1655 bacteria were able to grow to high numbers in the presence of high numbers of

MG1655 Δ*edd** bacteria when both were fed simultaneously to mice (Fig. 4B) or when low numbers of MG1655 bacteria were fed to mice precolonized with MG1655 Δ*edd** (Fig. 5B). These data suggest that in the streptomycin-treated mouse, colonization resistance, at least as it applies to *E. coli* MG1655, has a primarily nutritional basis and is not due to antimicrobials in the intestine.

We do not know whether the MG1655 *flhDC* operon deletion mutants utilize carbon sources better than their parents as a result of release of repression of genes normally regulated by the FlhD$_2$/FlhC$_2$ regulatory complex (e.g., the complex is known to repress *gltA* [citrate synthase], *sdhCDAB* [succinate dehydrogenase], *mdh* [malate dehydrogenase], and *mglBAC* [galactose transport] [36, 37]) or because increased energy is available for other cellular processes in the absence of flagellar synthesis and rotation, which is estimated to be about 2% of the total that is normally consumed (24). In either case, it appears that at least one enteric pathogen also benefits from loss of FlhD, as it was recently reported that an *flhD* mutant of *S. 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 (40). Furthermore, nonmotile *E. coli* O157:H$^-$ 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* (31).

The IS*1* element in the regulatory region of the *flhDC* operon presumably directed the downstream deletions identified in MG1655* and MG1655 Δ*edd**, which then allowed the isolation of stable, nonmotile, better-colonizing mutants selected by the intestine. However, it is possible that commensal *E. coli* strains that lack insertion elements in the regulatory region of the *flhDC* operon can also become nonmotile and utilize carbon sources better in the intestine by down regulating expression of *flhD* and *flhC*, perhaps via one or more of the known negative regulators of the operon, which include LrhA, OmpR, and RcsB (12, 21, 22, 41), but retain motility and utilize carbon sources normally after growth in the laboratory. In fact, it has been reported that after growth in cecal mucus in vitro, both *E. coli* F-18, a human commensal strain, and an avirulent *S. enterica* serovar Typhimurium strain failed to tumble and swim but were motile upon subsequent growth in laboratory medium (25, 26). In this same vein, it has recently been shown that transcription of several *Campylobacter jejuni* flagellar genes was generally down regulated after 24 to 48 h in a rabbit ileal loop model (42). Despite these reports, it is important to emphasize that not all bacteria in the intestine benefit from becoming permanently nonmotile. In fact, stable nonmotile mutants of many enteric pathogens, including *C. jejuni*, have been reported to be impaired in both intestinal colonization and virulence (17).

In summary, in the present study, we present evidence that under the nutrient-limiting conditions in the mouse intestine, better-colonizing MG1655 mutants are selected with deletions in the regulatory region of the *flhDC* operon. The deletions render the mutants nonmotile and simultaneously make them able to grow faster than their parents on a number of sugars present in the mouse intestine and in cecal mucus in vitro. The selection of *E. coli* mutants better able to utilize sugars than their parents may play an important role in limiting the ability of

invading strains, either commensal or pathogenic, to colonize the intestine. It will be of great interest to examine whether the specific strategy described here is peculiar to MG1655 or is shared by other commensal and pathogenic strains of *E. coli*.

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