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# Genotype and Phenotypes of an Intestine-Adapted *Escherichia coli* K-12 Mutant Selected by Animal Passage for Superior Colonization<sup>∇†</sup>

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**We previously isolated a spontaneous mutant of *Escherichia coli* K-12, strain MG1655, following passage through the streptomycin-treated mouse intestine, that has colonization traits superior to the wild-type parent strain (M. P. Leatham et al., *Infect. Immun.* 73:8039–8049, 2005). This intestine-adapted strain (*E. coli* MG1655\*) grew faster on several different carbon sources than the wild type and was nonmotile due to deletion of the *flhD* gene. We now report the results of several high-throughput genomic analysis approaches to further characterize *E. coli* MG1655\*. Whole-genome pyrosequencing did not reveal any changes on its genome, aside from the deletion at the *flhDC* locus, that could explain the colonization advantage of *E. coli* MG1655\*. Microarray analysis revealed modest yet significant induction of catabolic gene systems across the genome in both *E. coli* MG1655\* and an isogenic *flhD* mutant constructed in the laboratory. Catabolome analysis with Biolog GN2 microplates revealed an enhanced ability of both *E. coli* MG1655\* and the isogenic *flhD* mutant to oxidize a variety of carbon sources. The results show that intestine-adapted *E. coli* MG1655\* is more fit than the wild type for intestinal colonization, because loss of FlhD results in elevated expression of genes involved in carbon and energy metabolism, resulting in more efficient carbon source utilization and a higher intestinal population. Hence, mutations that enhance metabolic efficiency confer a colonization advantage.**

*Escherichia coli*, often one of the first bacteria to colonize infants (14, 16, 44, 45), is present in the gastrointestinal (GI) tracts of all humans (14, 53) and is found in the GI tracts of most mammals (8, 13, 35, 47). *E. coli* resides and grows within the nutrient-rich mucus layer of the intestine, which provides many needed biosynthetic precursors, carbon sources, and electron acceptors (7, 15, 19, 28, 29, 39, 40). Colonization of the GI tract requires bacteria to effectively compete for these nutrients and maintain a growth rate at least equal to the 2-h turnover rate of the intestinal contents (9–11). We have shown that of the many traits that possibly make *E. coli* such a remarkably successful intestinal colonizer, competition for carbon sources plays an important role (1, 7, 15, 29).

It has long been known that passage through the animal intestine can enhance virulence of certain pathogens (21, 26, 27), but the enhancement of colonization by animal passage only recently has been studied. A few years ago, we isolated a spontaneous mutant of wild-type *E. coli* K-12, strain MG1655, following passage through the intestine of a streptomycin-treated mouse, which is a better colonizer than its wild-type parent strain (31). This intestine-adapted strain (*E. coli* MG1655\*) grew faster on several different carbon sources than

the wild type and was nonmotile due to deletion of the *flhD* gene (31). FlhDC, the master regulator of flagellar synthesis, also has been implicated in control of several metabolic gene systems (34). Thus, there were two possible explanations for why *E. coli* MG1655\* grows faster than the wild-type on several carbon sources. First, the *flhD* mutant might save energy by not making flagella and not turning the flagellar motor. Second, loss of *flhD* could cause upregulation of genes unrelated to motility, i.e., catabolic genes, that enhance colonization fitness (19). We subsequently obtained evidence that supported both of these possibilities (19). In the wild-type *E. coli* MG1655 strain, an insertion element (*IS1*) immediately upstream of *flhD* increases promoter activity, causing hypermotility and strong repression of some carbohydrate metabolism genes (2). This *IS1* element appears to be a hot spot for deletion of *flhD* in the mouse intestine, as we isolated a number of closely related *E. coli* MG1655 derivatives following animal passage, each with deletions of various lengths, beginning at the same base position immediately downstream of the *IS1* element (19). In addition, pathogenic *Escherichia coli* O157:H<sup>-</sup> strains with deletions knocking out FlhDC have been associated with up to 40% of patients with hemolytic uremic syndrome in Germany (41). Thus, it seemed important to further characterize the intestine-adapted *E. coli* MG1655\* strain.

There are seven *IS1*, seven *IS2*, five *IS3*, one *IS4*, 11 *IS5*, one *IS150*, three *IS186*, four *IS30*, one *IS600*, and two *IS911* elements in the *E. coli* MG1655 genome (3, 51). Since IS elements are hot spots for bacterial speciation and genome evolution (32), we cannot rule out the possibility that other changes across the *E. coli* MG1655 genome occurred during passage

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† Supplemental material for this article may be found at <http://iai.asm.org/>.

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TABLE 1. Bacterial strains used in this study

<i>E. coli</i> strain	Relevant characteristic(s)	Source or reference
MG1655	Wild-type (CGSC no. 7740)	<i>E. coli</i> Genetic Stock Culture Collection, Yale University
MG1655 Str <sup>r</sup> strain	Spontaneous streptomycin-resistant mutant of MG1655	7
MG1655 Str <sup>r</sup> Nal <sup>r</sup> strain	Spontaneous nalidixic acid-resistant mutant of MG1655 Str <sup>r</sup>	21
MG1655*	Intestine-adapted mutant of the MG1655 Str <sup>r</sup> Nal <sup>r</sup> strain	19
MG1655 $\Delta$ <i>flhD::cat</i> strain	$\Delta$ ( <i>flhD-flhC</i> ):: <i>cat</i> deletion mutant of the MG1655 Str <sup>r</sup> strain, carrying the Cm resistance cassette	31
MG1655 $\Delta$ IS1 strain	<i>ISI::pflhD</i> deletion of the MG1655 Str <sup>r</sup> strain	19

through the mouse GI tract. As deletion of certain *E. coli* regulatory genes (e.g., *fmr*, *crp*, *creB*) can result in unintended secondary deletions of *flhD* (22), it also seems possible that the inverse of this scenario could happen. For these reasons, we were interested in determining the extent of changes to the *E. coli* MG1655\* genome and whether or not the *flhD* deletion alone is sufficient to explain its enhanced colonization fitness.

We have further characterized *E. coli* MG1655\* using several high-throughput genomic analysis approaches and now show that the loss of *flhD* is sufficient to confer a colonization advantage in streptomycin-treated mice. Our results show that the intestine-adapted *E. coli* MG1655\* strain is more fit than the wild type for intestinal colonization, because loss of FlhD causes elevated expression of genes involved in carbon and energy metabolism, resulting in enhanced metabolic efficiency, which is pivotal for success in host colonization.

#### MATERIALS AND METHODS

**Bacterial strains and growth conditions.** Bacterial strains were derived from the *E. coli* MG1655 Str<sup>r</sup> (streptomycin-resistant) strain, a K-12 strain, and are listed in Table 1. The intestine-adapted *E. coli* MG1655 Str<sup>r</sup> strain (MG1655\*), obtained as a colony present in mouse feces at 15 days postfeeding (see the description of the mouse colonization experiment, below), was isolated and characterized as described previously (31). It has a 500-bp deletion immediately downstream of *ISI* in the regulatory region of *flhD*, extending into *flhD* (31). The *flhD* deletion strain was constructed by using the allelic replacement method of Datsenko and Wanner (12) and contains a 546-bp deletion beginning immediately downstream of *ISI* in the regulatory region of *flhD* and extending into *flhD*. The deletion was replaced with a chloramphenicol resistance (Cm<sup>r</sup>) cassette, as described previously by Chang et al. (7). The mutation was verified by PCR analysis and by DNA sequencing of the PCR products (4). Cultures were routinely grown at 37°C on Luria-Bertani (LB) medium (33).

**Mouse colonization experiment.** Streptomycin-treated mice were used to study competition in the intestine between streptomycin-resistant wild-type *E. coli* and the isogenic *flhD* mutant strain. We previously described our use of this animal model in detail (30). 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 (31). Following 18 h of starvation for food and water, the mice were fed 1 ml of 20% (wt/vol) sucrose containing 10<sup>5</sup> CFU of *E. coli* strains grown overnight on LB medium. After the mice ingested the bacterial suspension, both the food (Harlan Teklad mouse and rat diet; Madison, WI) 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 at the indicated times. Mice were housed individually in cages without bedding and placed in clean cages at 24-h intervals. Fecal samples (1 gram) were therefore no older than 24 h. Each fecal sample was homogenized in 10 ml of 1% Bacto tryptone (Difco) and diluted in the same medium. Dilutions were plated on lactose MacConkey agar containing streptomycin sulfate (100  $\mu$ g/ml) and nalidixic acid (Nal; 50  $\mu$ g/ml) to distinguish the wild-type *E. coli* MG1655 Str<sup>r</sup> Nal<sup>r</sup> strain from the *E. coli* MG1655 Str<sup>r</sup> *flhD::cat* strain, which was plated on MacConkey agar containing streptomycin sulfate (100  $\mu$ g/ml) and chloramphenicol (30  $\mu$ g/ml). The nalidixic acid and chloramphenicol resistance markers used to distinguish the wild-type from the

$\Delta$ *flhD* mutant have no effect on its colonization ability (39). The colonization experiments were run in triplicate, and the data were averaged (9 mice).

**Whole-genome sequencing.** Genomic DNA from *E. coli* MG1655\* for pyrosequencing was isolated from a shaking culture grown overnight at 37°C in LB medium by using a DNeasy kit (Qiagen). The methods we used to generate shotgun and paired-end DNA libraries for the 454/Roche GS-FLX-Titanium sequencer are described in detail elsewhere (63). Briefly, the genomic DNA (50  $\mu$ g) was sheared in a nebulizer at 30 lb/in<sup>2</sup> to generate a fragment length of 500 to 800 bp for the shotgun library. To generate the paired-end library, genomic DNA was sheared in a HydroShear (GeneMachines) apparatus to generate DNA fragments with an average length of 2.2 kb. DNA fragment length was assessed by analysis on a Caliper AMS-90 using an SE30 DNA LabChip. For generation of the shotgun library, the ends of the fragmented DNA were repaired by using DNA polymerase, the 454 adapters were ligated onto the fragment ends, and the ends were repaired a second time to obtain blunt-ended DNA, followed by quantification, dilution, and amplification via emulsion PCR, in preparation for pyrosequencing (63). The paired-end library utilized a common central linker to identify potential paired ends, which were purified and circularized, followed by nebulization, as described above, prior to emulsion PCR amplification in preparation for sequencing (63). The genome sequence was obtained from the two libraries by massively parallel pyrosequencing with a 454/Roche GS-FLX-Titanium system (36). Data were collected from four sequencing runs, which yielded  $\sim 1.6 \times 10^6$  reads that passed the 454/Roche filters, with an average length of 233 bases, for a total of  $\sim 3.7 \times 10^8$  bases. The coverage depth, based on the size of the parent genome (3), was 75-fold.

**Sequence assembly and validation.** To search the *E. coli* MG1655\* genome for mutations that may have been acquired during animal passage, the data were mapped to the published *E. coli* MG1655 RefSeq genome sequence (accession number NC\_000913) by using GS Reference Mapper software (454/Roche), which assembled 1,491,412 reads, resulting in a total of 52 contigs ranging in length from 103 to 406,010 bases, with an average contig length of 88,609. Using this assembly strategy, repeat regions result in gaps (36), which explains why the genome was not closed completely.

**Microarray experiments and statistics.** The microarray protocols and data analysis used in this study were described previously (15). Briefly, total RNA was extracted from mid-logarithmic-phase cultures grown on MOPS [3-(*N*-morpholino)propanesulfonic acid]-defined medium containing 0.2% (wt/vol) glucose as the sole carbon source (43) with gyratory shaking at 250 rpm. Culture samples were diluted into DNA-RNA Protect (Sierra Diagnostics) to inhibit RNA degradation, and the RNA was purified using RNeasy minikits with the optional DNase treatment (Qiagen). The RNA was converted to cDNA using Superscript II (Invitrogen) and random hexamers, according to the manufacturer's specifications. The resulting cDNA was fragmented and biotinylated (Enzo Kit, Roche Diagnostics), according to the Affymetrix prokaryotic labeling protocol. Biotinylated samples were hybridized to Affymetrix GeneChip custom microarrays for 16 h at 60°C. The custom GeneChips contained probe sets for all genes on the *E. coli* MG1655 genome. Hybridized arrays were stained using the Affymetrix protocol (ProkGE\_WS2v2\_450). Biological replicates of cultures were used for all microarrays. After hybridization, microarrays were scanned and .cel files were further analyzed as described previously (15). Briefly, the raw .cel files were processed in RMA (quartile normalization) (25) by using a Bioconductor R package (affy, version 1.8.1, 3 September 2005) with an optional filter set to analyze only the subset of probe sets corresponding to MG1655 genes and intergenic regions. Microarrays of *E. coli* MG1655\* and the *E. coli* MG1655  $\Delta$ *flhD::cat* strain grown on glucose were used as the experimental condition, and the MG1655 Str<sup>r</sup> Nal<sup>r</sup> strain grown on glucose was used as the control

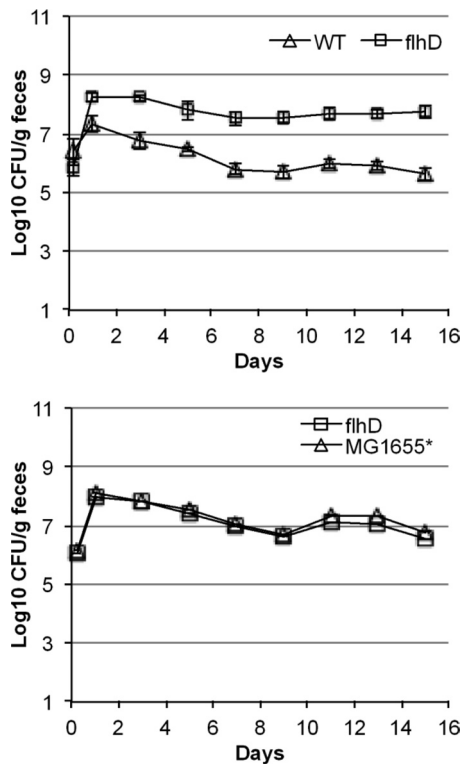


FIG. 1. (Top) Competition of the *E. coli* MG1655 *flhD* mutant against the *E. coli* MG1655 wild type in the streptomycin-treated mouse intestine. (Bottom) Competition of the *E. coli* MG1655 *flhD* mutant against *E. coli* MG1655\*. Error bars show the standard errors of the means for 9 animals.

condition. Fisher’s exact test was used to determine whether the modest gene expression changes examined were statistically relevant.

**BiOLOG GN2 MicroPlate assays.** BiOLOG assays involved an experiment design developed by Ihssen and Egli (24) and used with some modifications (61). Briefly, overnight cultures grown in MOPS minimal glucose medium were used to inoculate fresh medium (1% inoculum). When the cultures reached an optical density at 600 nm of 0.3, chloramphenicol (30 µg ml<sup>-1</sup>) was added and the cells were washed three times in MOPS minimal medium lacking a carbon source and containing chloramphenicol. The washed cells were resuspended to an optical density at 600 nm of 0.3 and inoculated into the microplate. The BiOLOG GN2 microplates were placed in an OmniLog plate reader at 37°C and read every 15 min for 24 h. Each experiment was duplicated on separate days, and the data were analyzed.

**Accession numbers.** The sequence data are available at GenBank, accession number CM000960 AEFEG1000000. The microarray data are available at the GEO repository, accession number GSE25106.

**RESULTS**

**Colonization fitness advantage of the *E. coli* MG1655 *flhD* mutant.** We previously showed that *E. coli* MG1655\* grows 6 to 23% faster on several different sugars and about 15% faster on cecal mucus, as sole carbon and energy sources, than its wild-type parent. Moreover, *E. coli* MG1655\* has a colonization advantage over the wild type (31). Subsequently, *E. coli* MG1655\* was found to have an *flhD* deletion. When tested for its growth rate on mannose and ribose, we found that *E. coli* MG1655 *flhD* grows as rapidly as *E. coli* MG1655\* under these conditions (31). We also found that the *E. coli* MG1655 *flhD* mutant grows faster than its wild-type parent on a mixture of 10

different sugars known to be present in mouse cecal mucus (19). Therefore, it was of interest to determine whether the *E. coli* MG1655 *flhD* mutant is a better colonizer than the wild type. To test this possibility, groups of 3 CD-1 mice were treated for 24 h with streptomycin (5 mg/ml) in their drinking water to open a niche for experimental colonization, and then the mice were fed 10<sup>5</sup> CFU each of the *E. coli* MG1655 *flhD*::*cat* Str<sup>r</sup> strain and the *E. coli* MG1655 Str<sup>r</sup> Nal<sup>r</sup> wild type; the Cm<sup>r</sup> cassette was used to enumerate the *E. coli* MG1655 *flhD*::*cat* population, and the Nal<sup>r</sup> cassette was used to count the wild-type population. As shown in Fig. 1, the *E. coli* MG1655 Str<sup>r</sup> *flhD* mutant had about a 2-log (100-fold)-higher population than the *E. coli* MG1655 Str<sup>r</sup> Nal<sup>r</sup> wild type. From this result, we concluded that mutation of *flhD* is sufficient to confer a colonization advantage that is analogous to that of the intestine-adapted strain (31).

**Pyrosequencing of the intestine-adapted *E. coli* MG1655\* genome.** Wild-type *E. coli* MG1655 has an *IS1* element within its *flhD* regulatory region that increases transcription of the *flhDC* operon, resulting in increased production of the flagellar master regulator, which causes wild-type *E. coli* MG1655 to be hypermotile (2). Following passage through the streptomycin-treated mouse intestine, the majority of intestine-adapted derivative strains are nonmotile (19). Previously, we used PCR mapping to identify on the *E. coli* MG1655\* genome an ~500-bp deletion that begins immediately downstream of the *insB* gene of the *IS1* element and extends into the *flhD* gene (31). PCR mapping of 10 additional intestine-adapted derivatives of *E. coli* MG1655 found that each contains a deletion that begins at the same locus proximal to the *IS1* element and extends at various lengths (~100 to 6,100 bp) into and beyond the *flhDC* operon (19). Since IS elements can promote genome rearrangements, they are hot spots for mutation (32, 42, 62), and there are approximately 50 transposon-related loci on the *E. coli* MG1655 strain (51), which represents significant potential for genetic change. Therefore, we sequenced the genome of intestine-adapted *E. coli* MG1655\* to determine whether the *flhD* mutation is solely responsible for its colonization fitness advantage or if additional mutations occurred during animal passage.

To sequence the *E. coli* MG1655\* genome, we used a massively parallel pyrosequencing strategy that is now routine in genome centers (63). The pyrosequencing summary statistics are shown in Table 2. We obtained 75-fold coverage of the genome and used GS Reference Mapper to align the reads against the *E. coli* MG1655 reference genome (RefSeq accession number NC\_000913). The resulting assembly consists of

TABLE 2. Summary statistics for pyrosequencing of *E. coli* MG1655\*

Parameter	Value
No. of instrument runs.....	4
No. of high-quality reads.....	1,586,667
Average read length (bases).....	233
Total no. of contigs.....	52
Avg (range) contig size (bp).....	88,609 (103 to 406,010)
Total no. of assembled reads in contigs.....	1,491,412
Total no. of sequenced bases in contigs.....	369,376,896



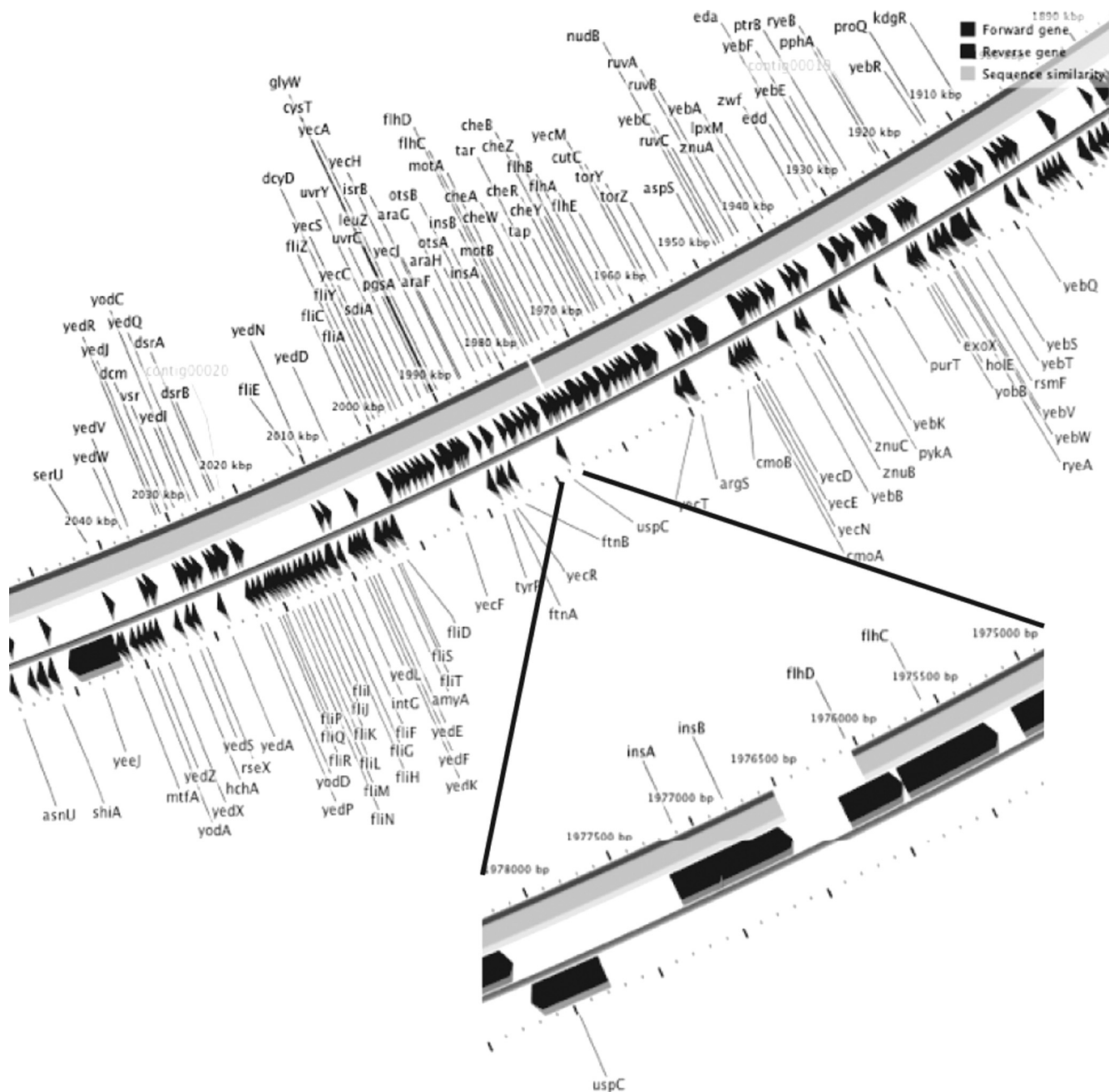


FIG. 2. *E. coli* MG1655\* genome sequence displayed in the Circular Genome Viewer. The 500-bp deletion at the *flhD* locus is shown at the higher resolution. No other deletions were found (see the text for details).

52 contigs averaging 88,609 bp and ranging between 103 and 406,010 bp long. The *flhD* gene was the only single-copy gene containing a deletion in the *E. coli* MG1655\* genomic sequence (Fig. 2), and it maps to a 500-bp gap between contigs 19 and 20 that begins 15 bp downstream of the *insB* gene of the ISI element, extends across the *flhD* regulatory region, and removes the first 195 bp of the *flhD* coding sequence. Given that PCR mapping places the common endpoint of other *flhD* deletions obtained by animal passage in the same region adjacent to the *insB* locus (19), we propose that the corresponding gap in the *E. coli*

MG1655\* genome sequence is due to a deletion promoted by the ISI element and selected because it confers a colonization fitness advantage. The remaining 51 gaps in the *E. coli* MG1655\* genome sequence cover regions containing genes that are highly repeated and include 26 insertion sequence genes (*ins*), 18 rRNA genes (*rrs* or *rrl*), 5 transposon-related function genes (*rhs*), and 2 prophage genes (*ydfJ*); each gap represents sequences that are identical to between 2 and 11 other loci, making them impossible to close by pyrosequencing. The Newbler assembler software typically does not include high-copy-number repeat sequences and thus results in gaps

TABLE 3. Summary of minor genotypic changes<sup>a</sup> on *E. coli* MG1655\* genome

Gene affected	Mutation	Base position <sup>b</sup>
<i>ylbE</i>	A to G substitution	547,964
<i>ylbE</i>	G insertion	547,835
<i>gatC</i>	CC insertion	2,171,386
<i>gyrA</i>	C to A substitution	2,337,184
<i>rpsL</i>	T to G substitution	3,472,447
<i>glpR</i>	G deletion	3,558,478
<i>ppiC-yifN</i> intergenic	C to T substitution	3,957,957
<i>glpP</i>	CG insertion	4,294,404

<sup>a</sup> Insertions or deletions of one or two bases and single-base substitutions.

<sup>b</sup> Relative to *E. coli* MG1655 reference sequence (accession no. NC\_000913).

that represent repeats, despite the inclusion of paired-end reads (36, 63). Nevertheless, the *E. coli* MG1655\* genome assembly is of very high quality, as roughly one-half of the regions containing IS elements were “closed.” Thus, while we are unable to rule out the possibility that additional deletions promoted by an insertion sequence did not occur, we can state with absolute certainty that *flhD* is the only nonrepeat gene that was deleted on the *E. coli* MG1655\* genome.

Our search for minor genotypic changes in the *E. coli* MG1655\* genome, by comparison to the *E. coli* MG1655 reference genome, was facilitated by having high-quality reads and 75-fold coverage. A total of 8 possible mutations were identified: four were single-base substitutions, three were insertions of 1 or 2 bases, and one was a single-base deletion. These data are shown in Table 3. We closely examined the eight putative mutations and considered their potential involvement in colonization. Two changes were found in the *yblE* coding sequence, and another was found in the intergenic region between *ppiC* and *yifN*. The former is a pseudogene, and the latter is unlikely to affect gene function; therefore, these three putative mutations are unlikely to impact colonization fitness. We found a single-base substitution in *gyrA* that converts the aspartate at codon 87 to tyrosine. This mutation, which is known to confer fluoroquinolone resistance on a clinical isolate of *E. coli* (10), is almost certainly the genotype responsible for the Nal<sup>r</sup> phenotype of the *E. coli* MG1655 parent strain of *E. coli* MG1655\*. We also found the substitution mutation in the *rpsL* gene that renders the *E. coli* MG1655 parent strain resistant to streptomycin, as we previously reported (30). We then focused attention on the three genotypic changes that were identified within coding sequences of genes involved in metabolism. A 2-base deletion was identified in the *gatC* gene, which encodes a component of the phosphoenolpyruvate (PEP)-dependent phospho-transferase system for galactitol uptake. We previously established that galactitol is not used by *E. coli* MG1655 to colonize streptomycin-treated mice (15). A single-base deletion was identified in the *glpR* gene, which encodes the regulator of the glycerol-3-phosphate pathway. Loss of the GlpR repressor would cause increased expression of this pathway, and indeed *glpT* and *glpC* were significantly induced in *E. coli* MG1655\* compared to in the wild type (see below), but other members of the GlpR regulon were repressed, so it is not clear how these differences could provide a colonization advantage over the wild type. We previously established that glycerol-3-phosphate is not used by *E. coli*

MG1655 to colonize streptomycin-treated mice (7). Lastly, we identified a 2-base insertion in the *glpP* gene that would inactivate the proton symporter for glutamate and aspartate (60). However, it is unlikely that transport function is lost, as *E. coli* also has the *gltIJKL*-encoded ABC uptake system for amino acids in addition to the *gltS*-encoded glutamate transporter.

To test directly the hypothesis that the genotypic changes to the *E. coli* MG1655\* genome, other than the *flhD* deletion, did not contribute to its colonization advantage, we fed *E. coli* MG1655\* and the *E. coli* MG1655 *flhD* mutant to mice. If the two strains were found to cocolonize, this would exclude the possibility that mutations on the *E. coli* MG1655\* genome other than an *flhD* mutation were responsible for its colonization advantage. As expected, we show in Fig. 1 that the two strains cocolonized, which indicates that *E. coli* MG1655\* and the *E. coli* MG1655 *flhD* mutant are identical in their ability to compete in the mouse intestine.

In summary, sequence analysis of the *E. coli* MG1655\* genome failed to reveal any mutations that could possibly cause the observed colonization advantage of this intestine-adapted strain, aside from the previously identified *flhD* deletion. Since the data shown in Fig. 1 are consistent with the hypothesis that an isogenic *flhD* mutant has a colonization advantage over *E. coli* MG1655 analogous to that of the intestine-adapted strain (31), we conclude that the *flhD* deletion is solely responsible for the colonization fitness advantage of *E. coli* MG1655\*.

**Transcriptome analysis of intestine-adapted *E. coli* MG1655\*.** The primary role of FlhDC is to act as the master regulator of flagellum biogenesis (34). The FlhDC regulatory network is known to overlap with several other regulatory networks (9, 17, 38, 46, 54, 56). For example, the high motility of *E. coli* MG1655 appears to be caused by a  $\sigma^{54}$  (sigma-N) promoter in the *insB-5* gene of the *ISI* element upstream of *flhDC*, suggesting a connection between control of nitrogen starvation and motility (66). In addition, the FlhDC master regulon controller is linked to catabolite repression control (57), adaptation to growth on acetate or propionate catabolism (48), and sugar acid catabolism (49, 55). Previously, we established that intestine-adapted *E. coli* grows 6 to 23% faster on several sugars (31), suggesting the possibility that *flhD* deletion strains express sugar catabolism genes at higher levels than wild-type *E. coli*. To interrogate the transcriptome of *E. coli* MG1655\* and to compare it to the wild-type and *flhD* deletion strains, cultures were grown to mid-logarithmic phase on MOPS minimal glucose medium. We routinely use these conditions to maximize reproducibility and to ensure that the cultures are in steady state (6, 58, 61). While the glucose causes catabolite repression, commonly chosen alternative growth conditions, such as on Luria broth (amino acids) or MOPS glycerol minimal medium, do not achieve steady-state, balanced growth (11). Aerobic growth conditions were chosen as being closest to the microaerobic conditions that *E. coli* encounters in the intestine. We previously established that *E. coli* must respire both oxygen and nitrate to compete successfully in the intestine (28), which indicates that the intestine is neither fully aerobic nor anaerobic and the oxygen tension *in situ* might fluctuate. Thus, anaerobic growth conditions are not physiologically relevant to colonization. Therefore, we chose to culture *E. coli* cells for transcriptome analysis on minimal glucose medium with aeration, which provides reproducible, steady-

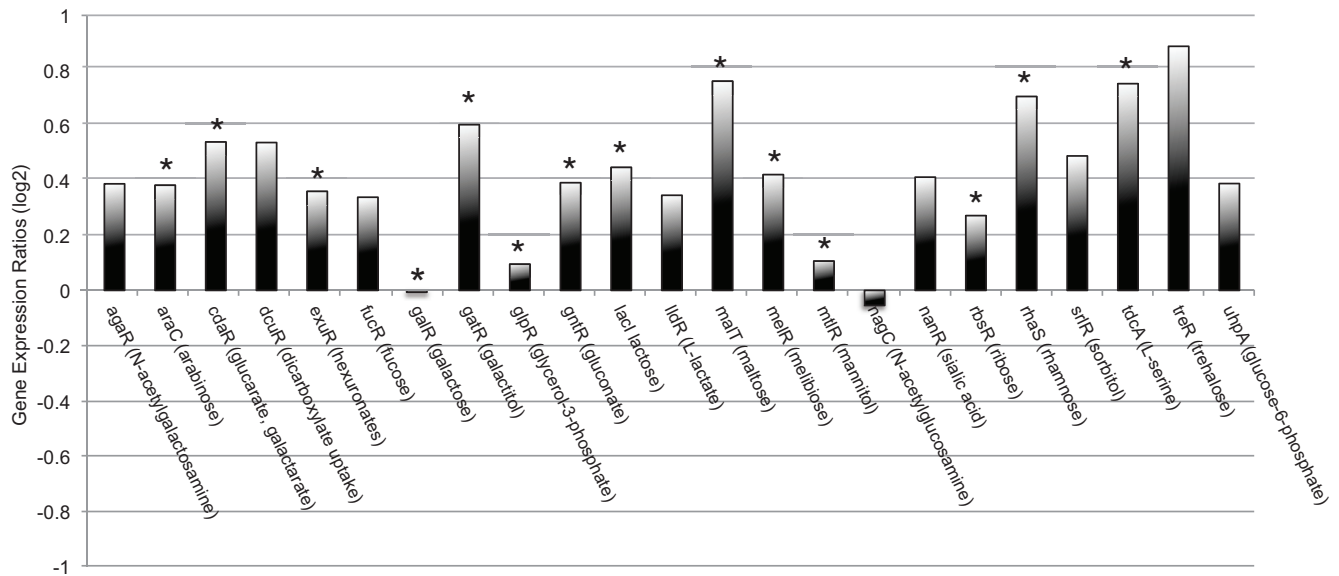


FIG. 3. Average regulon gene expression for carbon catabolism genes in *E. coli* MG1655\* by comparison to the wild type. An online tool called E-ring (<http://genexpdb.ou.edu/ering/>) was used to average gene expression for genes involved in carbon metabolism when grown on glucose. *E. coli* regulons are represented on the x axis by the regulator for each carbohydrate that *E. coli* can metabolize. The y axis represents the average log<sub>2</sub> expression ratio obtained by averaging the expression ratios for each gene within the given regulon. \*, *P* values of <0.005 in Fisher's exact test.

state growth conditions. Total RNA was extracted, labeled, and hybridized to a custom Affymetrix DNA microarray. The resulting transcriptome data were analyzed on an in-house bioinformatics platform, as described previously (7, 15), and used to address two basic questions. First, is expression of the sugar catabolism gene systems in the intestine-adapted strain consistent with the faster growth of the strain on several sugars and on cecal mucus? Second, is the *flhD* deletion alone sufficient to explain the enhanced catabolic capacity of *E. coli* MG1655\*?

**Induced catabolic regulon expression in the intestine-adapted strain.** To address the possibility that differences in its transcriptome cause *E. coli* MG1655\* to grow faster on several sugars, we evaluated the expression levels of carbon catabolism gene systems by comparison to the wild-type parent strain, when grown on glucose minimal medium. We focused on 23 carbohydrates that were previously tested for their ability to support intestinal colonization (1, 7, 15, 29) or showed more rapid growth of *E. coli* MG1655\* *in vitro* (31). The transcriptional network database, RegulonDB, was used to define genes within the corresponding 23 catabolic regulons (52). The regulons vary 10-fold in the numbers of members, ranging from 2 for the TreR (trehalose) regulon to 20 for the NagC (*N*-acetylglucosamine) regulon, and each of the corresponding 164 catabolic genes is represented in the data set only once. To quantify the differences in expression of the catabolic regulons between *E. coli* MG1655\* and the wild type, the expression ratios of the individual genes in each regulon were averaged, as shown in Fig. 3. All but two of the regulons (GalR [galactose] and NagC) were more highly expressed in the intestine-adapted strain. Overall, the average log<sub>2</sub> expression ratio of the 23 regulons was 0.41 higher (1.3-fold) in the intestine-adapted strain than in the wild type. Catabolite repression appeared to

play an important role in the increased expression of catabolic genes in *E. coli* MG1655\* when growing on glucose, since the average log<sub>2</sub> expression ratios of the 23 regulons of cells grown on mannose, which is far less catabolite repressing (37), was -0.10 (data not shown). The modest yet consistently higher expression of catabolic regulons appeared to be significant, as the average log<sub>2</sub> expression ratio for all known regulons (RegulonDB) was 0.06, with 100 regulons expressed higher in *E. coli* MG1655\* and 72 regulons expressed higher in the wild type (data not shown). The high incidence of elevated expression of catabolism genes extended beyond the 23 sugar regulons to include all 65 regulons that are involved in nutrient degradation (average log<sub>2</sub> expression ratio = 0.36), as opposed to the remaining 107 regulons that are not involved in degradation (average log<sub>2</sub> expression ratio = -0.11) (see Table S1 in the supplemental material). Thus, a simple transcriptome comparison of *E. coli* MG1655\* and its wild-type parent revealed a general, modest increase in the transcript levels of genes involved in nutrient uptake and catabolism, including the 23 regulons of interest.

In situations where a transcriptome experiment is designed to identify relatively small gene expression differences in a data set where very few genes exceed statistically significant thresholds, generally accepted statistical metrics are ineffectual, including the 2-fold difference rule of thumb (64) or differences greater than 2 standard deviations from the mean of the expression ratios (11, 59). In the present case, for the entire data set the standard deviation from the mean of the log<sub>2</sub> expression ratios is 0.64, and only 22 genes exceeded this threshold by 2-fold (1.28), none of them from the 23 regulon set of interest (see Fig. S1 in the supplemental material). When the genes are considered individually, there are some regulons in which all members have a positive expression ratio and others in which



some members have positive and others negative expression values (see Fig. S1). For all of these reasons, a traditional statistical approach cannot be used to establish significance in the data set. Therefore, we turned to an overrepresentation analysis, where lists of genes, predefined by RegulonDB, are analyzed to see which regulons are represented more than would be expected by chance (5). A similar approach based on overrepresentation of gene ontology annotations in microarray data has been successful (20). To evaluate the statistical probability that specific transcription factors are responsible for the observed transcriptional differences between the *E. coli* MG1655\* and wild-type strains, we applied Fisher's exact test, employing a two-tailed hypergeometric distribution to identify enriched transcription factors in RegulonDB that are overrepresented in the transcription data. The most overrepresented transcription factor of genes that exceeded the positive threshold was Crp (catabolite repression control). This was not surprising, since all but 13 genes in 2 of the 23 regulons under consideration also are under Crp control. Among the remaining 147 genes listed in RegulonDB as being Crp activated, 14 out of 23 of the corresponding transcription factors have *P* values of <0.005, as determined by Fisher's exact test (Fig. 3). Thus, we conclude that the probability of involvement of most of the carbon catabolism transcription factors exceeds the 5% confidence level for control of the modestly elevated transcript levels, under catabolite-repressing growth conditions, for the corresponding catabolic gene systems.

**The transcriptomes of *E. coli* MG1655\* and the  $\Delta$ *flhDC* strain are essentially identical.** The most prominent feature of the *E. coli* MG1655\* transcriptome experiments is substantially decreased expression of flagellar genes (see Fig. S2 in the supplemental material), particularly genes in the *flg* and *fli* operons. This result is consistent with the results of two related transcriptome analyses, published by others (50, 65), of *flhD* strains constructed for the purpose of characterizing the FlhDC and FliA regulons. To test the hypothesis that the *flhD* deletion in *E. coli* MG1655\* is solely responsible for the transcription patterns detailed above, we constructed an isogenic strain in the same wild-type *E. coli* MG1655 genetic background by replacing the *flhD* allele with a *Cm<sup>r</sup>* cassette. Based on the above results, we would expect that the transcriptomes of the two strains are essentially identical. We tested this hypothesis by using Fisher's exact test to analyze the regulatory networks that contributed the most significant differences between the *E. coli* MG1655\* and *E. coli* MG1655 *flhD* mutant strains, in comparison to the wild-type control arrays. The *P* values of eight representative regulons, four above and four below the threshold value used in the analysis (1.7 linear fold change), are shown in Table 4. In each case, the *P* value is below the cutoff that indicates a 5% confidence level (*P* < 0.005). As shown in Fig. S3 in the supplemental material, the average gene expression ratios for these regulons are essentially identical. The conclusion that the transcriptomes of *E. coli* MG1655\* and the *E. coli* MG1655 *flhD* mutant are essentially the same is further supported by the correlation values between the arrays. The correlation between the *E. coli* MG1655\* and *E. coli* MG1655 *flhD* arrays was 0.962, which compares favorably to the correlation between replicate control arrays (wild type) of 0.964 (data not shown). The correlations between the control and *E. coli* MG1655\* and between

TABLE 4. Fisher's exact test<sup>a</sup> showing regulatory networks affected in *E. coli* MG1655\* and the *E. coli* MG1655 *flhD* mutant compared to wild-type *E. coli* MG1655

Threshold	Regulon	Result for:	
		MG1655*	<i>flhD</i> mutant
Above	Crp	1.89E-03 <sup>a</sup>	1.53E-05
	Fnr	4.01E-06	3.80E-05
	GadX	1.37E-03	2.58E-04
	IHF	4.67E-07	3.65E-03
Below	FlhDC	1.28E-04	2.39E-06
	Fur	3.34E-06	1.35E-05
	PurR	4.81E-07	6.46E-15
	TyrR	1.66E-03	7.12E-04

<sup>a</sup> *P* value cutoff of 0.005 corresponds to 5% confidence level.

the control and the *E. coli* MG1655 *flhD* mutant were both 0.929 (data not shown). Thus, microarray comparison of *E. coli* MG1655\* and the *E. coli* MG1655 *flhD* mutant grown under the same conditions indicates that their transcriptomes essentially are identical, with no features of the *E. coli* MG1655\* transcriptome that would indicate obvious regulatory differences that could have been caused by unidentified mutations. We conclude that the *flhD* deletion acquired by *E. coli* MG1655\* during animal passage is responsible solely for its transcriptional phenotype: expression of catabolic gene systems is modestly, yet broadly and consistently, elevated in the intestine-adapted strain.

**Catabolome of an intestine-adapted strain compared to that of the wild type grown in glucose.** To determine whether the changes in the transcriptome of *E. coli* MG1655\* were reflected by its capacity to metabolize the indicated substrates, we used Biolog technology to measure the oxidation rates of 95 different carbon sources. To ensure that the results of the carbon source oxidation experiment were comparable to the microarray results, cultures were grown on minimal glucose medium as described above for the microarray analysis. The *E. coli* MG1655\* and wild-type strains were essentially equal in the number of carbon sources utilized. However, we found that *E. coli* MG1655\* oxidized 24 carbon sources to a greater extent than the wild-type parent strain (see Fig. S4 in the supplemental material). The data also show that the overall extent of carbon source oxidation was 21% higher for *E. coli* MG1655\* (Fig. 4). Furthermore, the overall extent of carbon source oxidation was the same for *E. coli* MG1655\* and the *flhD* deletion strain. Lastly, we examined an MG1655 strain in which the *ISI* element was removed from the regulatory region of the *flhDC* promoter that is normally motile (19) and that has normal levels of FlhDC. We found the *ISI* deletion strain had an intermediate level of carbon source oxidation compared to that of *E. coli* MG1655\* and the wild type. Therefore, the overall extent of carbon source oxidation was highest in strains lacking FlhDC (the *E. coli* MG1655  $\Delta$ *flhDC* strain and *E. coli* MG1655\*) and lowest in the wild-type strain (*E. coli* MG1655), which is hypermotile and overexpresses FlhDC. Thus, we conclude that loss of FlhDC in intestine-adapted *E. coli* MG1655\* results in release of several carbon catabolism operons from FlhDC repression and thereby an enhanced carbon source oxidation phenotype.

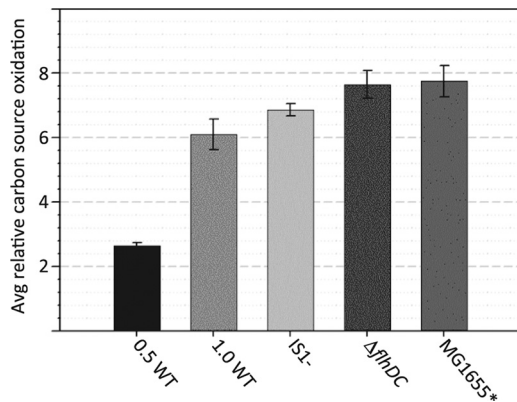


FIG. 4. Catabolome analysis of *E. coli* MG1655\* during growth on minimal glucose medium. Cells were grown to mid-log phase, harvested in chloramphenicol, loaded into BiOLOG GN2 microplates, and measured every 15 min for 24 h. The plot shows the overall extent of carbon source oxidation for wild-type *E. coli* MG1655 (“0.5 WT” indicates that one-half of the biomass was used compared to “1.0 WT”), strains deleted for *ISI* (*ISI*–) and *flhD* ( $\Delta flhDC$  mutant), and the intestine-adapted strain (MG1655\*). Error bars show the standard deviations.

## DISCUSSION

Passage of *E. coli* MG1655 through the GI tract of the streptomycin-treated mouse selected for a strain that differed from the wild type in being more fit for colonization (31). We subsequently found that the intestine-adapted strain, *E. coli* MG1655\*, acquired a deletion of the *flhD* gene that rendered it nonmotile and able to grow 6 to 23% faster than its wild-type parent in laboratory cultures on several sugars and on mouse cecal mucus (31). In a follow-up study, we found two reasons that *E. coli* MG1655\* was a more successful colonizer (19). First, mutational analysis showed that the *flhD* deletion apparently altered expression of genes not involved in motility that conferred enhanced colonization fitness. Second, the energy normally used by the wild type to synthesize flagella and power the flagellar motor was redirected to *E. coli* MG1655\* growth (19). To confirm that the observed colonization fitness advantage resulted from deletion of *flhD*, we sequenced the *E. coli* MG1655\* genome and used high-throughput approaches to characterize phenotypes relevant to its enhanced carbon catabolism. We found that the only *E. coli* MG1655\* genomic deletion partially removed the *flhD* gene immediately downstream of an *ISI* element that apparently promoted the deletion. We found two additional point mutations, one in a glycerol catabolism regulatory gene and the other in the *gatC* gene, which could inhibit galactitol uptake. However, after careful consideration of the available evidence, we concluded based on evidence discussed in Results above that neither of them would positively impact colonization and hence would not provide a selective advantage. No doubt, mutations are likely to occur spontaneously during the 180 generations spent in the intestine (2 h generation time for 15 days), but in the highly selective environment of the intestine, beneficial changes in genotype would be selected, and detrimental changes would be eliminated from the population, leaving only neutral mutations and those that are advantageous, such as *flhD*.

Based on the above results, we then investigated the nature

of the phenotypic changes promoted by deletion of *flhD* in an attempt to determine how they could provide a colonization advantage. Here, we observed that *E. coli* MG1655\* had a generally and modestly elevated (1.3-fold) expression of carbon and energy metabolism genes, together with a corresponding 21% overall increase in oxidation of 24 carbon and energy sources. These genome-wide increases are of the same order of magnitude as the 6 to 23% higher growth rates on several carbon sources previously observed in laboratory cultures (19), which we propose to result in the 100-fold-higher intestinal population during competition against the wild type (Fig. 1). Furthermore, we found no differences in the transcriptomes and catabolomes of *E. coli* MG1655\* and an *flhD* mutant constructed in *E. coli* MG1655. To search for clues as to how deletion of *flhD* could result in the observed differences, we compared our transcriptome data to previously reported studies (49) in which deletion of *flhD* caused downregulation of genes involved in sugar acid metabolism and decreased growth on gluconate, thought to be mediated by the redox sensor-encoding *aer* gene but by an unknown genetic mechanism (23). In distinct contrast, in our study we found that expression of sugar acid catabolism regulons was increased 1.3-fold, together with generally increased expression of a wide variety of sugar catabolism genes. The difference in growth conditions used in the two studies was revealing: the former results were obtained from cells grown on amino acids in Luria broth (49), and ours were from cells grown on glucose as the sole carbon source, as described above. As we noted, the vast majority of genes that showed altered expression in *E. coli* MG1655\* compared to that of the wild type are under Crp control. Moreover, when grown on glucose, the average  $\log_2$  expression ratio of the 23 regulons was 0.41, whereas for cells grown without significant catabolite repression on mannose, the average  $\log_2$  expression ratio was  $-0.10$ . These data suggest that catabolite repression control might be involved in the faster growth of the intestine-adapted strain in the intestine, but for reasons that are not clear. Thus, this transcriptome analysis demonstrates that deletion of *flhD* results in modestly higher expression of carbon and energy metabolism gene systems, which also was reflected physiologically in the carbon source oxidation patterns of cells grown under catabolite-repressing conditions. We therefore conclude that this increase in gene expression is directly responsible for the colonization advantage of *E. coli* MG1655\*.

In seeking to explain why *E. coli* MG1655\* is a better colonizer, we note that deletion of *flhD* does not expand its catabolic capacity to include additional carbon sources not used by the wild type. Therefore, it is more likely that the enhanced expression and activity of catabolic pathways is responsible for the colonization fitness advantage of the intestine-adapted strain. Conversely, in many cases, we found that genetic lesions in metabolic pathways resulted in decreased colonization fitness (7, 15). Not all of the mutations we previously tested completely eliminated growth on a particular carbon source. Some mutations removed one redundant pathway and hence slowed the growth rate on a particular carbon source, while other mutations caused sugar phosphates to accumulate, which led to growth inhibition. Since decreased growth rate of a mutant can cause a colonization defect, it stands to reason that increased growth rate could be selected by virtue of being advantageous. These concepts are central to a corollary of

Freter's nutrient-niche hypothesis (18), that the population of a species is proportional to the amount of its preferred carbon source. The results presented here indicate that more efficient carbon source utilization also results in a higher population size in the intestine. Mutations that enhance metabolic efficiency therefore confer a colonization advantage.

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#### REFERENCES

- Autieri, S. M., et al. 2007. L-Fucose stimulates utilization of D-ribose by *Escherichia coli* MG1655  $\Delta$ fucAO and *E. coli* Nissle 1917  $\Delta$ fucAO mutants in the mouse intestine and in M9 minimal medium. *Infect. Immun.* **75**:5465–5475.
- Barker, C. S., B. M. Pruss, and P. Matsumura. 2004. Increased motility of *Escherichia coli* by insertion sequence element integration into the regulatory region of the *flhD* operon. *J. Bacteriol.* **186**:7529–7537.
- Blattner, F. R., et al. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* **277**:1453–1474.
- Bodenteich, A., S. Chissoe, Y. F. Wang, and B. A. Roe. 1993. Shotgun cloning as the strategy of choice to generate templates for high-throughput dideoxynucleotide sequencing, p. 42–50. *In* J. C. Venter (ed.), *Automated DNA sequencing and analysis techniques*. Academic Press, London, United Kingdom.
- Boulesteix, A. L., G. Tutz, and K. Strimmer. 2003. A CART-based approach to discover emerging patterns in microarray data. *Bioinformatics* **19**:2465–2472.
- Chang, D. E., D. J. Smalley, and T. Conway. 2002. Gene expression profiling of *Escherichia coli* growth transitions: an expanded stringent response model. *Mol. Microbiol.* **45**:289–306.
- 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.
- Clapper, W. E., and G. H. Meade. 1963. Normal flora of the nose, throat, and lower intestine of dogs. *J. Bacteriol.* **85**:643–648.
- Clarke, M. B., and V. Sperandio. 2005. Transcriptional regulation of *flhDC* by QseBC and sigma (FlhA) in enterohaemorrhagic *Escherichia coli*. *Mol. Microbiol.* **57**:1734–1749.
- Conrad, S., et al. 1996. *gyrA* mutations in high-level fluoroquinolone-resistant clinical isolates of *Escherichia coli*. *J. Antimicrob. Chemother.* **38**:443–455.
- Conway, T., and G. K. Schoolnik. 2003. Microarray expression profiling: capturing a genome-wide portrait of the transcriptome. *Mol. Microbiol.* **47**:879–889.
- Datsenko, K. A., and B. L. Wanner. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U. S. A.* **97**:6640–6645.
- Duobos, R., R. W. Schaedler, and R. Costello. 1963. Composition, alteration, and effects of the intestinal flora. *Fed. Proc.* **22**:1322–1329.
- Escherich, T. 1988. The intestinal bacteria of the neonate and breast-fed infant. 1884. *Rev. Infect. Dis.* **10**:1220–1225.
- Fabich, A. J., et al. 2008. Comparison of carbon nutrition for pathogenic and commensal *Escherichia coli* strains in the mouse intestine. *Infect. Immun.* **76**:1143–1152.
- Favier, C. F., E. E. Vaughan, W. M. De Vos, and A. D. Akkermans. 2002. Molecular monitoring of succession of bacterial communities in human neonates. *Appl. Environ. Microbiol.* **68**:219–226.
- Francez-Charlot, A., et al. 2003. RcsCDB His-Asp phosphorelay system negatively regulates the *flhDC* operon in *Escherichia coli*. *Mol. Microbiol.* **49**:823–832.
- Freter, R. 1983. Mechanisms that control the microflora in the large intestine, p. 33–54. *In* D. J. Hentges (ed.), *Human intestinal microflora in health and disease*. Academic Press, Inc., New York, NY.
- Gauger, E. J., et al. 2007. Role of motility and the *flhDC* operon in *Escherichia coli* MG1655 colonization of the mouse intestine. *Infect. Immun.* **75**:3315–3324.
- Grossmann, S., S. Bauer, P. N. Robinson, and M. Vingron. 2007. Improved detection of overrepresentation of gene-ontology annotations with parent child analysis. *Bioinformatics* **23**:3024–3031.
- Herzberg, M., M. J. Jawad, and D. Pratt. 1965. Correlation of succinate metabolism and virulence in *Salmonella typhimurium*. *J. Bacteriol.* **89**:185–192.
- Hobman, J. L., et al. 2007. Comparative genomic hybridization detects secondary chromosomal deletions in *Escherichia coli* K-12 MG1655 mutants and highlights instability in the *flhDC* region. *J. Bacteriol.* **189**:8786–8792.
- Horne, S. M., K. R. Mattson, and B. M. Pruss. 2009. An *Escherichia coli* aer mutant exhibits a reduced ability to colonize the streptomycin-treated mouse large intestine. *Antonie Van Leeuwenhoek* **95**:149–158.
- Hisssen, J., and T. Egli. 2005. Global physiological analysis of carbon- and energy-limited growing *Escherichia coli* confirms a high degree of catabolic flexibility and preparedness for mixed substrate utilization. *Environ. Microbiol.* **7**:1568–1581.
- Irizarry, R. A., et al. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics (Oxford, England)* **4**:249–264.
- Jacobson, M. A., and I. S. Falk. 1927. Studies on respiratory diseases XXIX. The influence of anti-serum and of animal passage upon the virulence and electrophoresis of pneumococci. *J. Bacteriol.* **13**:191–201.
- Johnson, J. A., P. Panigrahi, and J. G. Morris, Jr. 1992. Non-O1 *Vibrio cholerae* NRT36S produces a polysaccharide capsule that determines colony morphology, serum resistance, and virulence in mice. *Infect. Immun.* **60**:864–869.
- Jones, S. A., et al. 2007. Respiration of *Escherichia coli* in the mouse intestine. *Infect. Immun.* **75**:4891–4899.
- Jones, S. A., et al. 2008. Glycogen and maltose utilization by *Escherichia coli* O157:H7 in the mouse intestine. *Infect. Immun.* **76**:2531–2540.
- Leatham, M. P., 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.
- Leatham, M. P., et al. 2005. 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.
- Leavis, H. L., et al. 2007. Insertion sequence-driven diversification creates a globally dispersed emerging multiresistant subspecies of *E. faecium*. *PLoS Pathog.* **3**:e7.
- Luria, S. E., and M. Delbruck. 1943. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* **28**:491–511.
- MacNab, R. M. 1996. Flagella and motility, p. 123–145. *In* F. C. Neidhardt et al. (ed.) *Escherichia coli* and *Salmonella*, 2nd ed., vol. 1. ASM Press, Washington, DC.
- Mansson, I. 1957. The intestinal flora in horses with certain skin changes; with special reference to the coliform microbes. *Acta Pathol. Microbiol. Scand. Suppl.* **119**:1–102.
- Margulies, M., et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
- McGinnis, J. F., and K. Paigen. 1973. Site of catabolite inhibition of carbohydrate metabolism. *J. Bacteriol.* **114**:885–887.
- Mercante, J., K. Suzuki, X. Cheng, P. Babitzke, and T. Romeo. 2006. Comprehensive alanine-scanning mutagenesis of *Escherichia coli* CsrA defines two subdomains of critical functional importance. *J. Biol. Chem.* **281**:31832–31842.
- Miranda, R. L., 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.
- Moller, A. K., 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.
- Monday, S. R., S. A. Minnich, and P. C. Feng. 2004. A 12-base-pair deletion in the flagellar master control gene *flhC* causes nonmotility of the pathogenic German sorbitol-fermenting *Escherichia coli* O157:H– strains. *J. Bacteriol.* **186**:2319–2327.
- Naas, T., M. Blot, W. M. Fitch, and W. Arber. 1995. Dynamics of IS-related genetic rearrangements in resting *Escherichia coli* K-12. *Mol. Biol. Evol.* **12**:198–207.
- Neidhardt, F. C., P. L. Bloch, and D. F. Smith. 1974. Culture medium for enterobacteria. *J. Bacteriol.* **119**:736–747.
- Nowrouzian, F., et al. 2003. *Escherichia coli* in infants' intestinal microflora: colonization rate, strain turnover, and virulence gene carriage. *Pediatr. Res.* **54**:8–14.
- Palmer, C., E. M. Bik, D. B. Digulio, D. A. Relman, and P. O. Brown. 2007. Development of the human infant intestinal microbiota. *PLoS Biol.* **5**:e177.
- Pesavento, C., et al. 2008. Inverse regulatory coordination of motility and curli-mediated adhesion in *Escherichia coli*. *Genes Dev.* **22**:2434–2446.
- Pesti, L. 1963. Qualitative and quantitative examination of the intestinal bacterium flora of healthy pigs. *Zentralbl. Bakteriol. Orig.* **189**:282–293.
- Polen, T., D. Rittmann, V. F. Wendisch, and H. Sahm. 2003. DNA microarray analyses of the long-term adaptive response of *Escherichia coli* to acetate and propionate. *Appl. Environ. Microbiol.* **69**:1759–1774.
- Pruss, B. M., 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.
- Pruss, B. M., X. Liu, W. Hendrickson, and P. Matsumura. 2001. FlhD/FlhC-regulated promoters analyzed by gene array and *lacZ* gene fusions. *FEMS Microbiol. Lett.* **197**:91–97.



51. **Riley, M., et al.** 2006. *Escherichia coli* K-12: a cooperatively developed annotation snapshot—2005. *Nucleic Acids Res.* **34**:1–9.
52. **Salgado, H., et al.** 2006. RegulonDB (version 5.0): *Escherichia coli* K-12 transcriptional regulatory network, operon organization, and growth conditions. *Nucleic Acids Res.* **34**:D394–D397.
53. **Savage, D. C.** 1977. Microbial ecology of the gastrointestinal tract. *Annu. Rev. Microbiol.* **31**:107–133.
54. **Shin, S., and C. Park.** 1995. Modulation of flagellar expression in *Escherichia coli* by acetyl phosphate and the osmoregulator OmpR. *J. Bacteriol.* **177**:4696–4702.
55. **Silverman, M., and M. Simon.** 1974. Characterization of *Escherichia coli* flagellar mutants that are insensitive to catabolite repression. *J. Bacteriol.* **120**:1196–1203.
56. **Simms, A. N., and H. L. Mobley.** 2008. PapX, a P fimbrial operon-encoded inhibitor of motility in uropathogenic *Escherichia coli*. *Infect. Immun.* **76**:4833–4841.
57. **Soutourina, O., et al.** 1999. Multiple control of flagellum biosynthesis in *Escherichia coli*: role of H-NS protein and the cyclic AMP-catabolite activator protein complex in transcription of the *flhDC* master operon. *J. Bacteriol.* **181**:7500–7508.
58. **Takagi, M., T. Tsuchiya, and M. Ishimoto.** 1981. Proton translocation coupled to trimethylamine N-oxide reduction in anaerobically grown *Escherichia coli*. *J. Bacteriol.* **148**:762–768.
59. **Tao, H., C. Bausch, C. Richmond, F. R. Blattner, and T. Conway.** 1999. Functional genomics: expression analysis of *Escherichia coli* growing on minimal and rich media. *J. Bacteriol.* **181**:6425–6440.
60. **Tolner, B., B. Poolman, B. Wallace, and W. N. Konings.** 1992. Revised nucleotide sequence of the *gluP* gene, which encodes the proton-glutamate-aspartate transport protein of *Escherichia coli* K-12. *J. Bacteriol.* **174**:2391–2393.
61. **Traxler, M. F., et al.** 2008. The global, ppGpp-mediated stringent response to amino acid starvation in *Escherichia coli*. *Mol. Microbiol.* **68**:1128–1148.
62. **Twiss, E., A. M. Coros, N. P. Tavakoli, and K. M. Derbyshire.** 2005. Transposition is modulated by a diverse set of host factors in *Escherichia coli* and is stimulated by nutritional stress. *Mol. Microbiol.* **57**:1593–1607.
63. **Wiley, G., et al.** 2009. Methods for generating shotgun and mixed shotgun/paired-end libraries for the 454 DNA sequencer. *Curr. Protoc. Hum. Genet.* **18**:18.1.
64. **Wren, J. D., and T. Conway.** 2006. Meta-analysis of published transcriptional and translational fold changes reveals a preference for low-fold inductions. *Omics* **10**:15–27.
65. **Zhao, K., M. Liu, and R. R. Burgess.** 2007. Adaptation in bacterial flagellar and motility systems: from regulon members to ‘foraging’-like behavior in *E. coli*. *Nucleic Acids Res.* **35**:4441–4452.
66. **Zhao, K., M. Liu, and R. R. Burgess.** 2009. Promoter and regulon analysis of nitrogen assimilation factor, sigma-54, reveal alternative strategy for *E. coli* MG1655 flagellar biosynthesis. *Nucleic Acids Res.* **38**:1273–1283.

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