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Comparison of Carbon Nutrition for Pathogenic and Commensal *Escherichia coli* Strains in the Mouse Intestine[▽]

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The carbon sources that support the growth of pathogenic *Escherichia coli* O157:H7 in the mammalian intestine have not previously been investigated. In vivo, the pathogenic *E. coli* EDL933 grows primarily as single cells dispersed within the mucus layer that overlies the mouse cecal epithelium. We therefore compared the pathogenic strain and the commensal *E. coli* strain MG1655 modes of metabolism in vitro, using a mixture of the sugars known to be present in cecal mucus, and found that the two strains used the 13 sugars in a similar order and cometabolized as many as 9 sugars at a time. We conducted systematic mutation analyses of *E. coli* EDL933 and *E. coli* MG1655 by using lesions in the pathways used for catabolism of 13 mucus-derived sugars and five other compounds for which the corresponding bacterial gene system was induced in the transcriptome of cells grown on cecal mucus. Each of 18 catabolic mutants in both bacterial genetic backgrounds was fed to streptomycin-treated mice, together with the respective wild-type parent strain, and their colonization was monitored by fecal plate counts. None of the mutations corresponding to the five compounds not found in mucosal polysaccharides resulted in colonization defects. Based on the mutations that caused colonization defects, we determined that both *E. coli* EDL933 and *E. coli* MG1655 used arabinose, fucose, and *N*-acetylglucosamine in the intestine. In addition, *E. coli* EDL933 used galactose, hexuronates, mannose, and ribose, whereas *E. coli* MG1655 used gluconate and *N*-acetylneuraminic acid. The colonization defects of six catabolic lesions were found to be additive with *E. coli* EDL933 but not with *E. coli* MG1655. The data indicate that pathogenic *E. coli* EDL933 uses sugars that are not used by commensal *E. coli* MG1655 to colonize the mouse intestine. The results suggest a strategy whereby invading pathogens gain advantage by simultaneously consuming several sugars that may be available because they are not consumed by the commensal intestinal microbiota.

Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a noninvasive enteric pathogen of humans. EHEC has many virulence factors, including adhesins (e.g., intimin), effector proteins (e.g., *espG*), and toxins (e.g., Shiga toxin), which are necessary to cause disease and likely contribute to the strain's extremely low infectious dose (27). However, the first step in EHEC infection, following ingestion, is colonization. To colonize the gastrointestinal (GI) tract, EHEC must overcome the barrier to infection imposed by the host and the intestinal microbiota, which include commensal *E. coli* strains. While many factors, such as innate immunity, inhibitory substances of microbial origin, and competition for adhesion sites, can be barriers to invading microorganisms, Freter postulated that competition for nutrients is the most important of these factors (17–19). Freter's nutrient-niche hypothesis states that for a species to be successful in the intestine, it must grow faster than the washout rate and better than all other competitors on one or on a small number of growth-limiting nutrients. Accordingly, EHEC must compete with the resident microbiota to acquire the nutrients it needs to grow from low to high numbers in the GI tract. While housekeeping functions are not virulence factors per se, carbon and energy source metabolism is considered to be essential during the early stages of many bacterial infections (9). How EHEC competes with the GI microbiota for nutrients during colonization remains an open question.

Much more is known about colonization of the intestine by commensal *E. coli* strains (7, 8, 29). Commensal *E. coli* cells are dispersed throughout the mucus layer overlaying the intestinal epithelium (35). Mutant cells that fail to penetrate or survive in or, most importantly, grow on mucus are unable to compete and cannot colonize the intestine (36). Mucus is composed of mucin, glycoproteins, glycolipids, and epithelial cell debris and is about 50% polysaccharides (for a review see reference 7). While *E. coli* genomes encode approximately 40 glycoside hydrolases (20), *E. coli* cannot degrade mucus polysaccharides (23) and is limited to growth on simple monosaccharides and disaccharides (34). Thus, we recently tested the hypothesis that *E. coli* K-12 grows on mucus-derived sugars in the intestine and found that seven fermentable carbohydrates each contribute to growth to various degrees during colonization (6). This finding

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implies that *E. coli* could cometabolize several nutrients in vivo. Supporting this idea of cometabolism is the fact that *E. coli* simultaneously utilizes a mixture of six sugars under nutrient-limiting conditions in vitro (32). Also, it is known that slow growth in carbon-limited chemostats and growth on increasingly poor quality carbon sources induce catabolic pathways for which the inducer is absent from the growth medium, acting as a possible carbon-scavenging mechanism (24, 33, 48). However, the extent to which these carbon-scavenging strategies are important for colonization of the intestine is unknown. Therefore, the ability of *E. coli* EDL933 to cometabolize a mixture of carbon sources for colonization and the identity of the carbon sources used in vivo are other open questions.

An exhaustive in vitro study of monocultures failed to reveal significant differences in carbon source utilization between commensal *E. coli* strains and pathogenic *E. coli* O157:H7 (12). Comparative genomics of *E. coli* O157:H7 (strain EDL933) and *E. coli* K-12 (strain MG1655) revealed no major differences in the gene systems that encode and regulate the pathways for carbon source utilization (39), although we previously noted the absence of two catabolic pathways for mucus-derived amino sugars (galactosamine and *N*-acetyl-galactosamine) in *E. coli* MG1655 (38). Nevertheless, *E. coli* EDL933 grows from low to high numbers in the presence of higher numbers of *E. coli* MG1655, suggesting that the two strains may compete for different nutrients in the intestine (35).

Since there is reason to believe that the competition for nutrients is important for the establishment of *E. coli* EDL933 in the mammalian intestine and since there appears to be no in vitro nutritional differences between *E. coli* strains in monocultures, we sought to determine if *E. coli* EDL933 and *E. coli* MG1655 compete for the same or utilize different carbon sources in vivo. The possibility that *E. coli* cometabolizes several sugars when presented with a complex mixture has not been tested, so we determined the order of sugar consumption for both *E. coli* EDL933 and *E. coli* MG1655 from a chemically defined culture medium containing 13 sugars previously identified in cecal mucus (6–8, 29, 38). The results indicate that *E. coli* EDL933 and *E. coli* MG1655 cometabolize these substrates in nearly the same order in vitro. However, a systematic genetic analysis indicates that the two strains utilize different carbon sources for colonization of the streptomycin-treated mouse, suggesting that the pathogen may establish infection by taking advantage of nutrients in the intestine that are not consumed by commensal *E. coli* and the normal microbiota.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains and plasmids used in this study are listed in Table 1. Strains were derived from *E. coli* MG1655 Str^r (streptomycin-resistant strain) (36) and *E. coli* EDL933 Str^r (35). The originally sequenced *E. coli* strain MG1655 is closely related to the human isolate K-12, having been cured only of lambda phage and the F plasmid (3). Strain EDL933 is the sequenced prototype strain of *E. coli* O157:H7 (39). Null alleles were constructed by using the allelic replacement method of Datsenko and Wanner (11), as described previously (6), such that target genes were deleted and replaced with kanamycin or chloramphenicol resistance cassettes, which served as selectable markers in the mouse colonization experiments described below. Single gene deletions began with the start codon and ended with the stop codon, and multiple gene deletions began with the start codon of the first gene deleted and ended with the stop codon of the last gene deleted. Strains containing multiple mutations were constructed by sequential allelic replacement; the first inserted cassette was removed with FLP recombinase (11), followed by allelic replacement(s) and removal of the insertion, as necessary, leaving the selectable marker in the last mutation made. Mutant strains were verified phenotypically and by DNA sequencing. To phenotype the strains, 3-(*N*-morpholino propanesulfonic acid (MOPS) defined medium (37) was used with a 0.2% carbon source unless otherwise specified, with gyratory shaking at 250 rpm. Cell growth was monitored spectrophotometrically at an optical density at 600 nm (OD_{600}). For determination of the strain's in vitro nutrient preference, cultures were grown in MOPS medium containing 0.015% each of gluconate, *N*-acetyl-neuraminic acid (NANA), galactose, mannose, *N*-acetyl-glucosamine (NAG), *N*-acetyl-galactosamine (GalNAc), glucosamine, glucuronate, galacturonate, fucose, ribose, L-arabinose, and maltose. Overnight cultures of cells grown on MOPS-NAG medium were used to inoculate the mixed carbon source cultures. At selected time points, culture medium samples were collected by filtration through 0.45-μm-pore filters, followed by heating to 60°C for 30 min to inactivate any extracellular enzymes. The samples were frozen until analyzed, as described below.

Sugar analysis. Reducing sugars were derivatized with 4-aminobenzonitrile (4-ABN) and analyzed with CE and UV detection, using a method adapted from Schwaiger et al. (46). Nonreducing sugars were separated and detected directly using CE coupled to mass spectrometry. A 1.0 M 4-ABN reagent was prepared by dissolving 0.12 g of 4-ABN in 2.0 ml of 1 M sodium cyanoborohydride in tetrahydrofuran and 104 μl of acetic acid (all reagents were purchased from Sigma-Aldrich Chemical). The 4-ABN reagent was protected from light and stored at 5°C. Derivatization was conducted by mixing 40 μl of the sample with 10 μl of the internal standard (1 mM allose) and 10 μl of the 4-ABN reagent in a screw-top centrifuge tube and heating the mixture for 4 h at 90°C. The derivatization mixture was diluted 1:10 in the running electrolyte, and CE was carried out by using an Agilent G1600 automated CE instrument. The separation capillary was 50-μm-internal-diameter bare fused silica with a bubble cell and had an effective length of 56 cm (Agilent Technologies). The sample was injected hydrodynamically, using 25 kPa s^{-1} pressure. The running electrolyte was 140 mM borate (pH 10.2), and the separation potential was +22 kV. The column temperature was maintained at 25°C. Prior to each injection, the capillary was flushed for 3 min with the running electrolyte. A postrun step included a 2-min rinse with 0.1 M NaOH and a 1-min rinse with MilliQ H_2O. The UV signal was collected at 280 nm, 10 nm bandwidth, using a 520-nm reference signal.

Nonreducing sugars were detected by mass spectrometry as $[M - H]^-$ or $[M + H]^+$ adducts for the acidic and basic sugars, respectively. The CE separation was achieved using an Agilent G1600 automated CE instrument coupled to an Agilent G1946A single quadrupole mass spectrometer using electrospray ionization and the Agilent coaxial sheath fluid CE-electrospray ionization-mass spectrometry interface. The separation capillary was 50 μm internal diameter, with a total length of 70 cm (Polymicro Technologies), using a voltage of +25 kV. Injection was hydrodynamic at 40 kPa s^{-1}. The running electrolyte for the analysis of NANA and gluconate was 100 mM borate (pH 9.0; adjusted with NH_4OH). For glucosamine, GlcNAc, and GalNAc, the running electrolyte was 50 mM NH_4OAc with 10 mM borate, adjusted to pH 9.0 with NH_4OH. Extracted ion electropherograms were used for each carbohydrate at the appropriate $[M - H]^-$ or $[M + H]^+$ *m/z*. Relative sugar concentrations were determined by dividing the peak area for each component by that of the internal standard (allose) peak area. All peak area ratios were normalized to the arbitrary value of 100 for the first time point, for comparison purposes. Three analytical replicates were obtained for each time point, and the entire growth experiment was duplicated.

Microarray analysis of *E. coli* MG1655 and *E. coli* EDL933. Total RNA from *E. coli* MG1655 Str^r Nal^r (nalidixic acid-resistant) or *E. coli* EDL933 Str^r Nal^r was extracted from 10 ml of cells grown to an OD_{600} of 0.4 on MOPS minimal medium containing glucose (0.2% [wt/vol]) or cecal mucus (10 mg ml^{-1}) as the sole carbon source, as described previously (6). Culture samples were diluted into DNA-RNA Protect (Sierra Diagnostics) to inhibit RNA degradation, and the RNA was purified using RNeasy mini-kits with optional DNase treatment (Qiagen). The RNA was converted to cDNA by first-strand synthesis, using Superscript II (Invitrogen) and random hexamers, according to the manufacturer's specifications. The resulting cDNA was fragmented and biotinylated (with an Enzo kit; Roche Diagnostics), according to an Affymetrix prokaryotic labeling protocol. Biotinylated samples were hybridized to Affymetrix GeneChip custom microarrays for 16 h at 60°C. Custom GeneChips contained probe sets for all genes on the genomes of *E. coli* MG1655 and *E. coli* EDL933. Hybridized arrays were stained using an Affymetrix protocol (ProkGE_WS2v2_450). Stained microarrays were scanned, and the raw data files (with the file extension.cel) were analyzed further using RMA processing with quartile normalization (25). Biological duplicate array data were averaged, and the transcriptome of cells grown on cecal mucus was compared to that of cells grown on glucose. We considered

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Genotype or phenotype	Source or reference
MG1655	Wild type (CGSC no. 7740)[a]	CGSC
MG1655 Str^r	Spontaneous Str^r	36
MG1655 Str^r Nal^r	Spontaneous Nal^r	36
EDL933	Wild-type O157:H7	Alison O'Brien
EDL933 Str^r	Spontaneous Str^r	35
EDL933 Str^r Nal^r	Spontaneous Nal^r	35
Derivatives of strain MG1655 Str^r		
araBAD[b]	Δ(*araB-araD*)::*cat*	This study
aspA	Δ*aspA*::*cat*	6
fucAO	Δ(*fucA-fucO*)::*kan*	6
galK	Δ*galK*::*cat*	This study
gatABC	Δ(*gatA-gatC*)::*cat*	This study
glpK	Δ*glpK*::*kan*	6
gntK idnK	Δ*gntK* Δ*idnK*::*cat*	30
lacZ	Δ*lacZ*::*cat*	This study
manA	Δ*manA*::*cat*	This study
mtlA	Δ*mtlA*::*cat*	This study
nagE	Δ*nagE*::*kan*	6
nanAT	Δ(*nanA-nanT*)::*kan*	6
rbsK	Δ*rbsK*::*kan*	6
srlAEB	Δ(*srlA-srlB*)::*cat*	This study
uxaB	Δ*uxaB*::*kan*	6
uxaC	Δ*uxaC*::*kan*	6
Multiple-mutation derivatives of strain MG1655 Str^{rc}		
fucAO rbsK		This study
fucAO rbsK manA		This study
fucAO rbsK manA araBAD		This study
fucAO rbsK manA araBAD nagE		This study
fucAO rbsK manA araBAD nagE galK		This study
Derivatives of strain EDL933 Str^r		
agaWEFA	Δ(*agaW-agaA*)::*cat*	This study
araBAD	Δ(*araB-araD*)::*cat*	This study
aspA	Δ*aspA*::*cat*	This study
fucAO	Δ(*fucA-fucO*)::*kan*	This study
galK	Δ*galK*::*cat*	This study
gatABC	Δ(*gatA-gatC*)::*cat*	This study
glpK	Δ*glpK*::*cat*	This study
gntK	Δ*gntK*::*cat*	This study
lacZ	Δ*lacZ*::*cat*	This study
manA	Δ*manA*::*cat*	This study
manXYZ	Δ(*manX-manZ*)::*cat*	This study
mtlA	Δ*mtlA*::*cat*	This study
nagE	Δ*nagE*::*cat*	This study
nanAT	Δ(*nanA-nanT*)::*cat*	This study
rbsK	Δ*rbsK*::*cat*	This study
uxaB	Δ*uxaB*::*cat*	This study
uxaC	Δ*uxaC*::*cat*	This study
Multiple-mutation derivatives of strain EDL933 Str^{rc}		
fucAO rbsK		This study
fucAO rbsK manA		This study
fucAO rbsK manA araBAD		This study
fucAO rbsK manA araBAD nagE		This study
fucAO rbsK manA araBAD nagE galK		This study
Plasmids		
pKD3	*bla cat*	11
pKD4	*bla kan*	11
pKD46	T^s Ap^r P_{araC}-λ-red recombinase	11
pCP20	T^s Ap^r FLP recombinase	11

[a] CGSC, *E. coli* Genetic Stock Culture Collection, Yale University.
[b] For simplicity, strains are named in the text by strain and mutation(s).
[c] See Materials and Methods for details on multiple-mutation constructions; genotypes are identical to those listed in this table for single mutations.

genes to be significantly induced if their expression ratio was more than 2 standard deviations above the mean of the expression ratios (50).

Mouse colonization experiments. The streptomycin-treated mouse is the model of choice for the study of colonization of the mouse large intestine by *E. coli* (7, 8, 29). Briefly, three CD-1 male mice, 6 weeks of age, were given drinking water containing streptomycin sulfate (5 g/liter) for 24 h, which opens the niche for *E. coli* by removing the resident facultative microbiota, while leaving the anaerobic microbiota essentially intact (21). Following this treatment, the mice were starved for food and water for 18 h and were then fed (per oral) approximately 10^5 CFU of both the wild-type and the mutant strains in 1 ml of 20% sucrose. The wild-type strains were *E. coli* MG1655 Str^r Nal^r (36) and *E. coli* EDL933 Str^r Nal^r (35); Nal^r was used to distinguish the wild type (reference strain) from the null allele mutants in fecal plate counts. After the bacterial suspension was ingested, food and streptomycin-water (5 g/liter) were restored, and fecal plate counts were determined at 5 h and 24 h, and on every other day thereafter for 15 days. Fecal samples were homogenized and diluted in 1% tryptone broth and plated on lactose-MacConkey agar containing either streptomycin (100 μg/ml) and nalidixic acid (50 μg/ml) to count the wild type or streptomycin and kanamycin (40 μg/ml) or chloramphenicol (30 μg/ml) to count the null allele mutants. Each colonization experiment was repeated on separate occasions, and the values shown represent the averages for six mice. The $\log_{10}$ mean number of CFU per gram of feces plus or minus the standard error for each strain in the mice was calculated for each time point. In all experiments, a difference between two strains of ≥10-fold CFU/g feces (1 order of magnitude) was statistically significant, i.e., had a P value of <0.05 with Student's t test (two-tailed with unequal variance). The limit of detection for fecal plate counts was 10^2 CFU/g feces.

Microarray data accession number. Microarray data were deposited in Array Express, accession no. E-MEXP-1177.

RESULTS

In vitro cometabolism of carbon sources in a complex mixture. *E. coli* EDL933 and *E. coli* MG1655 have essentially identical carbon utilization profiles in monocultures growing on individual sugars (12), with the exception of GalNAc and galactosamine, because the *agaWEFA* genes are deleted in K-12 strains (3). *E. coli* is known to cometabolize several sugars when examined in chemostats and shaken batch cultures (for a review, see reference 28). However, the preferred order of carbon source utilization from a complex mixture of mucus-derived sugars has not been studied. Previously, we determined the in vitro order of carbon source preference for *E. coli* MG1655 by comparing a series of cultures, each containing two carbon sources, and monitoring diauxic growth for the disappearance of sugars (6). We were concerned that this experimental strategy did not give a true reflection of substrate consumption from complex mixtures. Therefore, we grew *E. coli* EDL933 and *E. coli* MG1655 aerobically in batch cultures on MOPS minimal medium supplemented with 13 sugars (0.015% of each) known to be present in mucus (38). Since galactosamine and GalNAc are metabolized by the same metabolic pathway, only GalNAc was added to the medium. Maltose was included because we previously observed that maltose genes were induced in *E. coli* MG1655 when it was grown on mucus (6). The disappearance of each sugar was monitored by CE and UV detection and by CE coupled to mass spectrometry, as described in Materials and Methods.

The results of this experiment (Fig. 1) indicated that the two strains used the carbon sources in a similar, but not identical, order. The in vitro order of nutrient preference for *E. coli* EDL933 was NAG > gluconate > galactose > NANA > mannose > galacturonate = glucuronate = arabinose = ribose > glucosamine > maltose = fucose = GalNAc. The order of nutrient preference for *E. coli* MG1655 was NAG > gluconate > galactose > NANA > galacturonate = glucuronate = mannose = ribose > arabinose > glucosamine > maltose > fucose (GalNAc was unused). The most significant differences between the two strains were the earlier beginning of mannose and arabinose consumption by *E. coli* EDL933 and the sequential onset of maltose and fucose utilization with *E. coli* MG1655. Also evident, as shown in Fig. 1, are cascading carbon source utilization and overlapping metabolism. The data indicated that *E. coli* EDL933 and *E. coli* MG1655 used catabolite-repressing sugars first (i.e., NAG, gluconate and galactose [41]). Before these catabolite-repressing sugars were completely exhausted, cometabolism of the remaining sugars ensued (up to nine sugars at one time). Acetate was excreted and accumulated in the growth medium throughout batch growth, until it was consumed during the last one-half generation, while the least preferred sugars were being exhausted. This pattern of acetate excretion and reuptake is typical of *E. coli* overflow metabolism when the bacteria are growing on fermentable sugars (49). Thus, the data indicated that the growth of both the pathogenic and the commensal strains is characteristic of sugar fermentation under aerobic conditions, with little evidence of differences between the strains. Growth of the cultures throughout the experiment was logarithmic, with smooth transitions through sequential substrate catabolism.

***E. coli* EDL933 transcriptome during growth in cecal mucus.** We previously showed that *E. coli* EDL933 can grow in vitro on cecal mucus prepared from mice but cannot grow in the luminal content, suggesting that the bacteria colonize the mouse intestine by growing in the mucus layer that overlays the cecal epithelium (35). In the streptomycin-treated mouse, fluorescence in situ microscopy of cecal thin sections demonstrate that *E. coli* EDL933 grows primarily as single cells dispersed throughout the mucus layer (>90%) and also in the region adjacent to the epithelium (35). Mucus contains 13 monosaccharides (arabinose, fucose, galactose, gluconate, glucuronate, galacturonate, mannose, glucosamine, NAG, galactosamine, GalNAc, NANA, and ribose) that are made available to *E. coli* by host epithelial cell turnover and polysaccharide-degrading anaerobes (8). With this list of available sugars in mind, we previously determined the transcriptome of *E. coli* MG1655 grown on mucus to identify genes that might be important for colonization (6). Under these conditions, which are thought to mimic the nutrient availability in the intestine, the majority of induced gene systems in *E. coli* MG1655 involved metabolism, including catabolic pathways for seven of the monosaccharides listed above (fucose, gluconate, glucuronate, galacturonate, NAG, NANA, and ribose) (6). Therefore, as a starting point for the current investigation, we used a custom Affymetrix GeneChip to measure the transcriptome of *E. coli* EDL933 grown to an OD_{600} of 0.4 (mid logarithmic phase) on cecal mucus. The control culture in these experiments was grown on glucose as the sole carbon and energy source. The results showed that gene systems involved in catabolism of 7 of the 13 sugars in mucus were significantly induced in mucus-grown *E. coli* EDL933, including fucose, gluconate, glucuronate, galacturonate, GalNAc, NANA, and ribose (data not shown). We previously observed a more comprehensive induction of metabolic genes at this culture density; however, the expression of some genes involved in the catabolism of preferred carbon

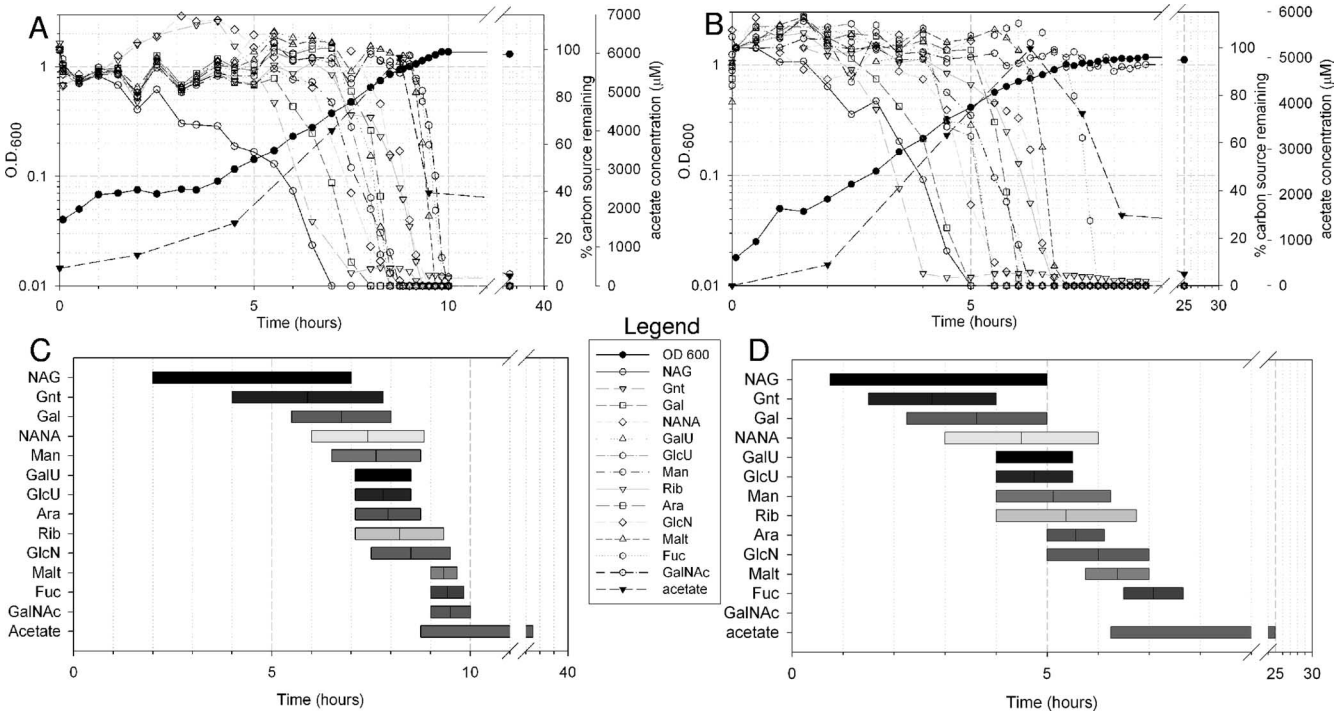

FIG. 1. Carbohydrate consumption by *E. coli* EDL933 (A and C) and *E. coli* MG1655 (B and D) of cultures grown in chemically defined medium containing a mixture of 0.015% each of 13 sugars. For panels A and B, the left axes show OD_{600}, the first right axis is the percentage of carbon source remaining, and the second right axis is acetate concentration (μM). The legend for carbon sources is shown. For panels C and D, the bars show the times that consumption of the indicated sugar began and was completed. The *x* axes of all plots indicate time (h). Abbreviations: gluconate (Gnt), galactose (Gal), galacturonate (GalU), glucuronate (GlcU), mannose (Man), ribose (Rib), arabinose (Ara), glucosamine (GlcN), maltose (Malt), fucose (Fuc), and *N*-acetyl-galactosamine (GalNAc).

sources may have declined, presumably because these carbon sources were present in low amounts and were consumed early in the logarithmic phase (6). In addition, we observed the induction of five gene systems in *E. coli* EDL933 (data not shown) and *E. coli* MG1655 (6) that are involved in the catabolism of glycerol, galactitol, lactose, mannitol, and aspartate. Thus, the gene systems induced by growth on mucus in *E. coli* EDL933 differed only slightly from that in *E. coli* MG1655 (6). Our previous systematic analysis of the colonization properties of catabolic mutants of *E. coli* MG1655 showed that some systems induced on mucus did not impact colonization and that others not induced on mucus did impact colonization (6). Based on these considerations, we decided to investigate each of the 18 carbon sources listed above (13 mucus-derived sugars plus 5 compounds corresponding to gene systems that were induced by mucus) as possible substrates for the colonization of *E. coli* EDL933.

***E. coli* EDL933 and *E. coli* MG1655 utilize different sugars in the mouse intestine.** To determine which of the 18 carbon sources support colonization of *E. coli* EDL933 in the intestine, a series of mutants were constructed with specific defects in carbon catabolism pathways (Table 1). The phenotype of each mutant was tested to ensure that the mutation specifically eliminated growth on the corresponding carbon source without affecting in vitro growth on other carbon sources (data not shown). The mutants were competed in vivo against the wild-type parent strain, as described previously for *E. coli* MG1655 (6). The streptomycin-treated mouse is the model of choice for studying bacterial carbon nutrition in the intestine (7, 8, 29). We have described this model in considerable detail previously (2, 26). We want only to stress here that the streptomycin-treated CD-1 mouse is used to study the competition for colonization between two strains, typically a mutant and its wild-type parent; it is not a model for pathogenesis, and none of the animals used in this study showed symptoms of disease at any time during the experiments. In this competitive fitness assay, mice are given, over the entire course of the experiment, streptomycin in their drinking water (5 g/liter), which selectively removes facultative anaerobes without affecting the overall populations of anaerobes in the intestine (21). Streptomycin-resistant *E. coli* mutants and wild-type strains are fed together at 10^5 CFU each to groups of three mice. Strains that cocolonize the animals at the same population level are considered equally fit; i.e., the inability to utilize the carbon source in question has no impact on colonization. Mutations that negatively impact the competitive fitness of the strain result in a colonization defect that is measured in logarithmic decreases in the relative population of the mutant compared to the wild type; i.e., catabolism of the corresponding carbon source by the wild type provides a competitive advantage over the mutant. Each experiment was replicated (with a total of six or more mice); all colonization differences exceeding 1 log (a 10-fold difference in population sizes) were statistically significant ($P < 0.05$) (26).

The results of a comprehensive mutagenic analysis to determine the relative fitness for the colonization of strains having

TABLE 2. Competitive colonization between sugar mutants and wild-type *E. coli* strains

Sugar	Mutation(s)	Log_{10} difference (CFU/g ± SEM) for *E. coli* EDL933 populations[a] on:		Log_{10} difference (CFU/g ± SEM) for *E. coli* MG1655 populations[a] on:	
		Day 1	Day 9	Day 1	Day 9
Arabinose	*araBAD*	0.4 ± 0.2	**1.4 ± 0.3**	0.1 ± 0.1	**2.2 ± 0.3**
Fucose	*fucAO*	0.4 ± 0.4	**2.8 ± 0.5**	**1.1 ± 0.2**	**1.8 ± 0.3**
Galactose	*galK*	0.9 ± 0.5	**2.9 ± 0.4**	0.4 ± 0.1	0.8 ± 0.2
Gluconate[b]	*gntK* (*idnK*)	0.3 ± 0.3	0.6 ± 0.5	0.6 ± 0.1	**1.4 ± 0.3**
Hexuronates[c]	*uxaC*	0.1 ± 0.1	**1.2 ± 0.2**	0.0 ± 0.4	0.0 ± 0.2
Galacturonate	*uxaB*	0.2 ± 0.2	0.8 ± 0.3	0.4 ± 0.3	0.8 ± 0.3
Mannose	*manA*	0.6 ± 0.2	**2.2 ± 0.9**	0.3 ± 0.5	0.1 ± 0.2
NAG	*nagE*	0.3 ± 0.3	**1.6 ± 0.4**	**1.2 ± 0.2**	**2.2 ± 0.3**
Glucosamine	*manXYZ*	0.6 ± 0.6	0.5 ± 0.3	ND[d]	ND
GalNAc and galactosamine	*agaWEFA*	0.6 ± 0.5	0.7 ± 0.4	Pathway missing[e]	Pathway missing[e]
NANA	*nanAT*	0.7 ± 0.2	−0.6 ± 0.2	**1.2 ± 0.3**	**3.3 ± 0.7**
Ribose	*rbsK*	0.2 ± 0.3	**1.1 ± 0.3**	0.0 ± 0.3	0.3 ± 0.2

[a] Each mouse was fed 10^5 CFU of a mutant strain and its wild-type parent. Values shown are the log_{10} differences in populations between the mutant and the wild types; i.e., the log_{10} CFU/g of feces for the mutant was subtracted from the log_{10} CFU/g of feces for the wild type. The average log_{10} differences ± standard error of the mean (SEM) of day 1 and day 9 data from at least six mice are shown. Colonization differences that exceeded 1 log and are statistically significant (Student's *t* test, $P < 0.05$) are shown in bold.

[b] *E. coli* EDL933 is deleted for *idnK*, which encodes the secondary gluconate kinase.

[c] The *uxaC* mutation blocks catabolism of both hexuronates, glucuronate, and galacturonate.

[d] ND, not determined.

[e] *E. coli* MG1655 is deleted for *agaWEFA*. *E. coli* EDL933 and MG1655, carrying mutations for the catabolism of aspartate (*aspA*), galactitol (*gatABC*), glycerol (*glpK*), lactose (*lacZ*), and mannitol (*mtlA*), did not have colonization defects (data not shown).

lesions in each of 18 different catabolic pathways are shown in Table 2. Mutation of the majority (9 out of 13) of the catabolic pathways for sugars known to be present in cecal mucus caused colonization defects in one or both of the strain backgrounds tested. In the pathogenic *E. coli* EDL933, mutation of 7 of the 13 catabolic pathways caused statistically significant defects in competition with the wild type in the maintenance stage (day 9 of the experiments). None of these mutations caused defects in the initiation stage of colonization by *E. coli* EDL933 (day 1). In the commensal strain, *E. coli* MG1655, mutation of five of the catabolic pathways caused statistically significant colonization defects in the maintenance stage, three of which were also defective in the initiation stage. Not all mutations tested caused colonization defects: mutation of nearly half (8 out of 18) of the catabolic pathways did not result in a colonization defect in either *E. coli* EDL933 or *E. coli* MG1655; none of the five catabolic pathways for the compounds not found in mucosal polysaccharides, but for which the corresponding gene systems were induced by growth on mucus (glycerol, galactitol, lactose, mannitol, and aspartate), provided a colonization advantage (as listed in the legend to Table 2; data not shown).

Mutants of both *E. coli* EDL933 and *E. coli* MG1655 that were unable to grow on arabinose, fucose, and NAG had colonization defects. With respect to differences between the pathogenic and the commensal strain, mutation of the pathways for galactose, hexuronates, mannose, and ribose caused colonization defects for *E. coli* EDL933 but not for *E. coli* MG1655, whereas mutation of pathways for gluconate and NANA caused colonization defects for *E. coli* MG1655 but not *E. coli* EDL933. Examples of in vivo carbon nutrition differences between *E. coli* EDL933 and *E. coli* MG1655 for galactose and NANA, respectively, are shown graphically in Fig. 2. We note that it is not possible to distinguish glucuronate catabolism by mutation (6, 38). The *uxaB* mutation that eliminates galacturonate catabolism did not have a statistically significant effect on colonization, and the *uxaC* mutation that prevents growth on both glucuronate and galacturonate was just over the significance limit, suggesting that catabolism of neither compound alone contributes significantly to colonization. To summarize these colonization assay results, commensal *E. coli* MG1655 appeared to use arabinose, fucose, gluconate, NAG, and NANA to colonize, whereas the pathogenic *E. coli* EDL933 appeared to use arabinose, fucose, galactose, mannose, NAG, and ribose to colonize the streptomycin-treated mouse.

Colonization defects in *E. coli* EDL933 are additive. We found that *E. coli* EDL933 metabolizes up to nine sugars simultaneously in vitro (Fig. 1), and at least 6 sugars contribute to its ability to colonize the mouse intestine (Table 2). This suggested that, in vivo, *E. coli* EDL933 might cometabolize several sugars to maintain colonization. To test this possibility, we constructed a series of mutant strains with defects in multiple catabolic pathways. To the strain that could not grow on fucose, we added a lesion in ribose catabolism, and to the strain that could not grow on fucose and ribose, we added a lesion in mannose catabolism, and so on, until we also removed the abilities to grow on arabinose, NAG, and galactose (Table 1). To compare the effects of these mutations on the pathogenic and the commensal backgrounds, we constructed a similar series of mutations in *E. coli* MG1655. Each of these mutations was competed in mice against their respective wild types, as shown in Fig. 3. By plotting the log difference between the *E. coli* EDL933 mutants and the *E. coli* EDL933 wild type, we observed that the colonization defects for each of these six mutations were additive, i.e., the larger the number of catabolic lesions, the larger the differences in population between the *E. coli* EDL933 mutants and the wild-type *E. coli* EDL933. While this relationship was linear in the *E. coli* EDL933 background, a very different result was obtained for *E. coli* MG1655.

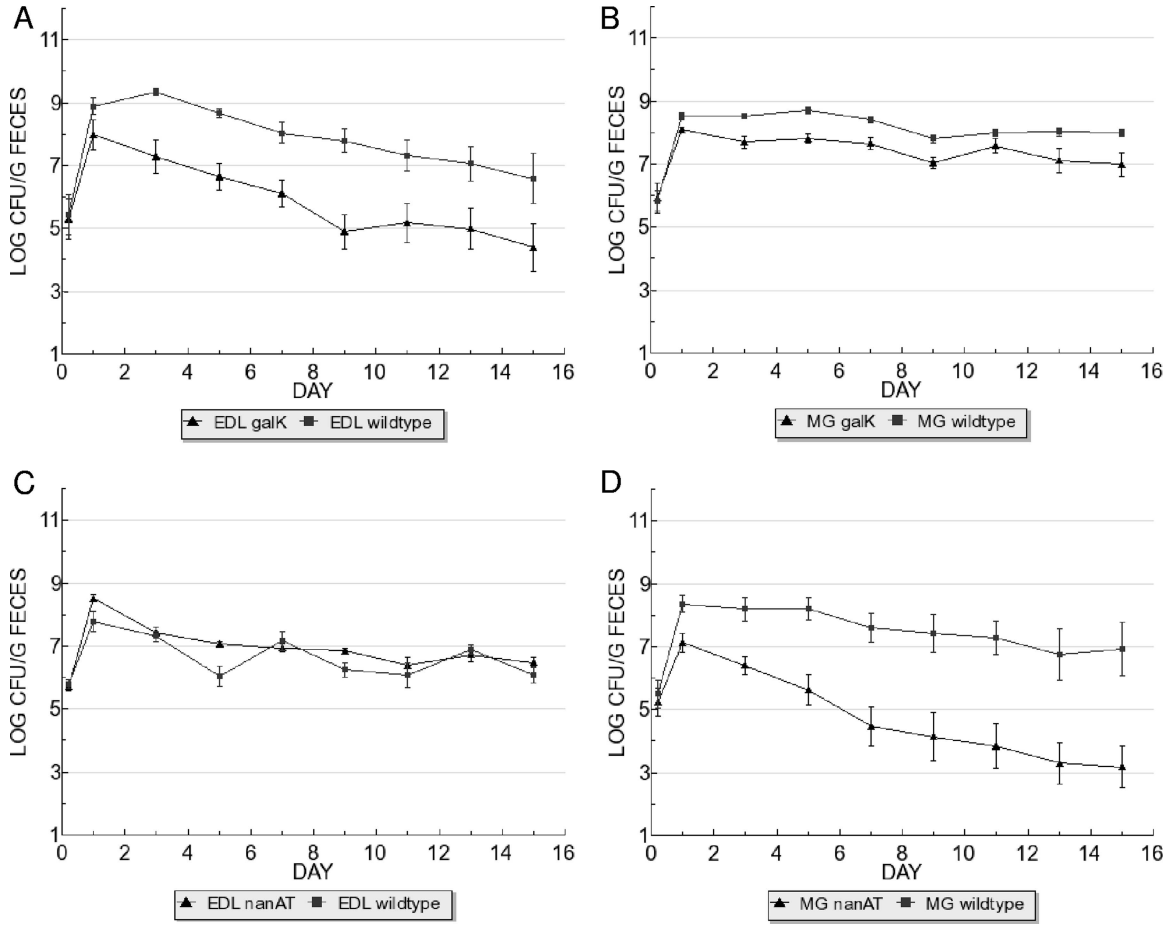

FIG. 2. Differences in catabolic pathways that caused colonization defects in *E. coli* EDL933 and *E. coli* MG1655. Mutation of the pathway for galactose catabolism (*galK*) caused a colonization defect in *E. coli* EDL933 (A) but not in *E. coli* MG1655 (B). Mutation of the pathway for NANA catabolism did not cause a colonization defect in *E. coli* EDL933 (C) but did in *E. coli* MG1655 (D).

In *E. coli* MG1655, there was a 2.1 log colonization defect with the *fucAO* mutant and a 4.4 log defect with the *fucAO rbsK* mutant, compared to defects of 2.5 log and 3.1 log, respectively, in the pathogen. We attribute this more dramatic difference in *E. coli* MG1655 to a metabolic switch that we described recently (2): the *E. coli* MG1655 *fucAO* mutant switches to ribose in the intestine, whereas the *fucAO rbsK* mutant cannot, causing a much-larger-than-expected colonization defect. The addition of a lesion in mannose metabolism to the latter strain had no effect on its competitiveness, but the addition of a fourth lesion, in arabinose, actually improved the colonizing ability of the quadruple mutant (*fucAO rbsK manA araBAD*) to a 3.1 log defect, suggesting an additional metabolic switch in this strain. A fifth lesion in NAG catabolism improved the colonizing ability of the quintuple mutant still further, i.e., a 1.9 log defect, suggesting yet another metabolic switch. The sixth lesion tested was in galactose catabolism, which resulted in a strain with a 5.2 log defect and suggested that one of the metabolic switches was to galactose, a sugar that *E. coli* MG1655 does not normally use in the mouse intestine (Table 2). The nature of these metabolic switches in the commensal strains is not well understood and is under investigation. What is clear from the results shown in Fig. 3 is that *E. coli* EDL933 does not exhibit these metabolic switches. Rather, the effects of multiple catabolic lesions in *E. coli* EDL933 are additive, indicating that the pathogen depends on up to six sugars, which it probably metabolizes simultaneously to support intestinal colonization.

DISCUSSION

In this study, we found that lesions in seven different catabolic pathways caused colonization defects in *E. coli* EDL933 when the mutants were competed against the wild type in mouse. Mutation of the galactose catabolic pathway had the largest relative impact on colonization fitness, followed by mutation of the pathways for fucose, mannose, NAG, arabinose, and ribose (Table 2). In addition, a mutation that prevented the growth of *E. coli* EDL933 on both glucuronate and galacturonate (*uxaC*) caused a minor but significant colonization defect, although there was not a significant defect for the lesion in galacturonate catabolism only (*uxaB*). The colonization by *E. coli* MG1655 was most affected by a lesion in the catabolism of NANA, followed in their relative impact by NAG, arabinose, fucose, and gluconate (Table 2). These results compare favorably with those from previously reported experiments in-

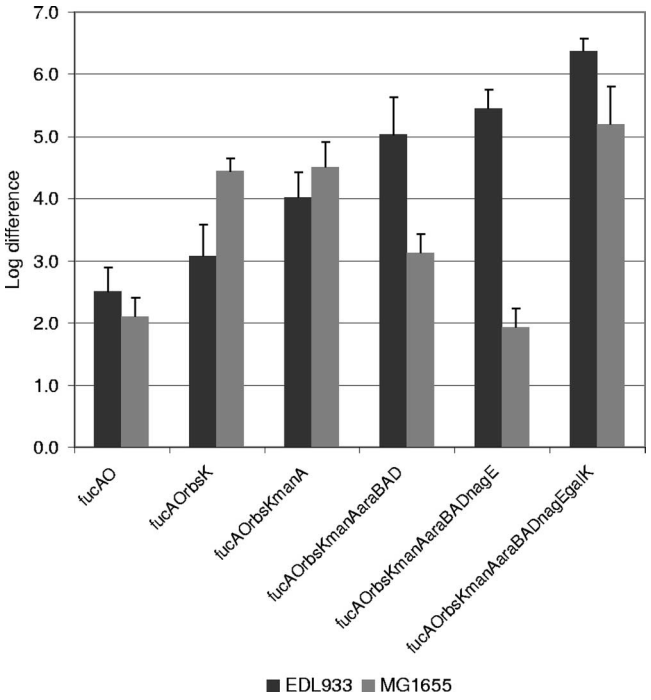

FIG. 3. Additive effect of colonization defects shown in *E. coli* EDL933. The effects of the series of mutations (shown in Table 2 and described in text) are shown on the *x* axis for *E. coli* EDL933 and *E. coli* MG1655 and are expressed as the log of the difference between the population sizes of the mutants in competition with their respective wild types at day 15 of the experiments (*y* axis).

dicating that catabolism of NAG, NANA, fucose, and gluconate is important for the colonization of *E. coli* MG1655 (6). It is necessary to revise our previous findings that glucuronate, mannose, and ribose catabolism mutants had minor colonization defects; according to results of the additional experiments shown in Table 2, catabolism of these three sugars did not significantly impact colonization by *E. coli* MG1655. Importantly, *E. coli* EDL933 appears to use three sugars that are not used by *E. coli* MG1655: galactose, mannose, and ribose (Fig. 2). Conversely, *E. coli* MG1655 uses two sugars that are not used by *E. coli* EDL933: NANA and gluconate. That the two strains prefer different sugars for colonization suggests a strategy for enteric pathogens, i.e., *E. coli* O157:H7, to invade the intestine and colonize in the presence of commensal *E. coli* strains. The nutrients that are not consumed by the commensal microbiota would be available for pathogens and might enable them to overcome colonization resistance and grow from low to high numbers to establish infection.

We previously reported that *E. coli* EDL933 is initially able to grow from low to high numbers in the presence of high numbers of *E. coli* MG1655 when the two strains are fed simultaneously to mice (35). The sugars preferred by *E. coli* EDL933 but not by *E. coli* MG1655 might support this initial population increase. However, *E. coli* EDL933 subsequently declines in numbers relative to those of *E. coli* MG1655, and *E. coli* EDL933 is unable to initiate colonization in mice that have been precolonized with *E. coli* MG1655 (35). Since the relative population of a species in the intestine depends on the available concentrations of its preferred nutrients (19), one explanation for the advantage of *E. coli* MG1655 over *E. coli* EDL933 could be that it has higher substrate affinities for the sugars that are used by both strains, which would force *E. coli* EDL933 to use less abundant nutrients. This possibility is supported by evidence that *E. coli* EDL933 attempts to switch from glycolytic to gluconeogenic substrates when it is competing with *E. coli* MG1655 (35). It will be interesting to measure the available concentrations of sugars in mucus prepared from streptomycin-treated mice and to determine the substrate affinities of different *E. coli* strains. It is also of interest to determine how well *E. coli* EDL933 can compete with other commensal *E. coli* strains and how the relative population sizes of the various strains correlate with differences in substrate affinities and nutrient preferences.

In conducting this study, it was important for each of the mutations to be specific for the carbon source in question. Otherwise, it would be difficult to ascribe direct cause and effect to metabolic lesions and corresponding colonization defects. For example, we reported previously that the mutation of the Entner-Doudoroff pathway affects growth on several carbon sources that do not use that pathway (30) and that mutations which cause the accumulation of certain intermediates of NAG metabolism can inhibit growth in the presence of NAG (6); in both cases, erroneous results were obtained for the colonization assays. We were therefore careful to characterize the phenotype of each of the strains used in this study to confirm that the metabolic lesion was specific to the corresponding carbon source(s) and that growth was not affected by the presence of that carbon source. Despite taking this precaution, it is possible for a mutation to affect colonization without impacting in vitro growth, as has been shown for the *galETKM* operon deletion in *E. coli* O157:H7, which has a rabbit colonization defect (22). Presumably, the *galETKM* mutant had no O antigen (not directly measured in this study) because the mutation of *galE* blocks conversion of UDP-glucose to UDP-galactose, which is a precursor of GalNAc, a constituent of the O157 antigen. For this reason, and to prevent the accumulation of potentially toxic phosphorylated intermediates (4, 16), we made a null mutation in the galactokinase gene, *galK*, which was designed not to affect *galE* expression; the *galK* mutation makes the strain phenotypically galactose-negative, but UDP-galactose can still be made from UDP-glucose. We also considered how the mutants used in our study might have impacted biosynthesis of the other three constituents of the O157 antigen, glucose, fucose, and *N*-acetylperosamine (40). UDP-glucose metabolism is not affected by any of the mutations we tested; GDP-fucose and GDP-perosamine are both made from GDP-mannose by pathways that are independent of either mannose or fucose catabolism (45, 47, 51). Thus, we are confident that none of the mutants we constructed for testing in mice was affected in its ability to make lipopolysaccharide.

The nutrient-niche hypothesis states that each species in the intestine must grow better than all others on one or a few limiting nutrients (19). Previously we suggested that this hypothesis is somewhat oversimplified and should be revised in consideration of the metabolic diversity of individual species (6). The experiments described here provide additional evidence that *E. coli* can cometabolize several sugars. For in vitro results, we observed the simultaneous metabolism of as many

as nine sugars (Fig. 1). The ability of *E. coli* to simultaneously metabolize up to six sugars in a chemostat is well documented (15, 24, 31, 32). In vivo results show that the mutation of pathways for several different sugars causes colonization defects (Table 2). Thus, it is likely that several substrates are cometabolized in vivo. Moreover, several catabolic lesions are required to eliminate the ability of *E. coli* EDL933 to compete with its wild-type parent (Fig. 3). In addition, *E. coli* EDL933 (*fucAO rbsK manA araBAD nagE*) can colonize at wild-type levels when it alone is fed to mice (data not shown), indicating that its catabolic capacity extends beyond these five most-preferred compounds. Thus, we conclude that the nutrient-niche hypothesis should be revised further to include the concept that metabolic flexibility provides a competitive advantage in the intestine, i.e., the catabolic capacity of an intestinal species is tuned to variations in nutrient availability. In this light, pathogens might be especially good at taking advantage of available nutrients that are not consumed by the normal microbiota.

Growth of *E. coli* is limited to monosaccharides, disaccharides, one trisaccharide, and maltodextrins up to 20 glucose units in length (34). While there are several glycoside hydrolases carried by the *E. coli* EDL933 and *E. coli* MG1655 genomes, none of them are secreted enzymes that can hydrolyze complex polysaccharides; there are no significant differences between pathogenic and commensal *E. coli* strains in this regard (20). Phenotypically, *E. coli* cannot degrade the complex polysaccharides found in mucus (23). It is the dominant anaerobes that degrade polysaccharides in the intestine (5, 10, 13, 23, 44). At least some minor members of the microbiota cross-feed on sugars released from polysaccharides by the anaerobes (14, 43). There is recent evidence that vancomycin-resistant *Enterococcus* spp. colonize the intestine by growing on monosaccharides released by the breakdown of complex polysaccharides (42). Thus, as we have suggested previously, it appears that *E. coli* grows on simple sugars released by anaerobic digestion (38). While the current study focused on the in vivo catabolism of monosaccharides, we showed that at least one disaccharide, lactose, is not important for colonization. A more extensive analysis of disaccharide and maltodextrin utilization by *E. coli* is indicated.

Aside from the obvious differences between *E. coli* EDL933 and *E. coli* MG1655 in the genetic presence or absence of specific catabolic pathways (i.e., GalNAc), there is no evidence that the global regulation of metabolism differs in the two genetic backgrounds (39), nor are there significant differences in their growth phenotypes on single carbon sources (12). Thus, we were intrigued to find that the relative impact of catabolic lesions on colonization did not strictly follow the in vitro preference for these sugars, i.e., for *E. coli* EDL933, lesions in galactose and fucose catabolism had larger defects in vivo than those in mannose and NAG, despite being consumed in vitro in the order NAG, galactose, mannose, fucose (Fig. 1). For comparison, in *E. coli* MG1655, the impact of a lesion in fucose catabolism was larger than that for gluconate, and the lesion in NANA catabolism was larger than that for NAG, despite being consumed in vitro in the order NAG, gluconate, NANA, fucose (Fig. 1). The physiology underlying the differences between in vivo versus in vitro nutrient preference is not clear. What is clear is that the environment of the intestine, whether it is related to host- or microbiota-derived factors, must affect the selection of nutrients by colonized *E. coli*, since these differ significantly from those preferred in vitro. Moreover, we found that lesions in the six catabolic pathways that support colonization by *E. coli* EDL933 are additive, i.e., each additional loss of metabolic capacity in the mutant strains resulted in a larger colonization defect (Fig. 3). The commensal *E. coli* MG1655, on the other hand, did not respond additively to multiple catabolic lesions, but rather, the catabolic mutant series behaved as if the sequential loss of particular catabolic pathways led to metabolic switches such as the one we recently described for fucose-dependent stimulation of ribose catabolism (2). Perhaps the relative importance of fucose utilization by both *E. coli* strains in vivo, despite being a low priority substrate in vitro, relates to this presumed "signaling" role of fucose. The results suggest that there may be a fundamental difference in the way *E. coli* strain EDL933 and MG1655 perceive and alter their nutrient choices in response to the intestinal environment. Unraveling the signals that affect *E. coli* nutrient choice in vivo and the mechanism(s) by which *E. coli* modifies its catabolic capacity in response to these signals will likely lead to a new understanding of the food web and ecology of the GI tract. It is interesting to speculate on a fundamental difference between pathogenic and commensal *E. coli* with regard to the value of metabolic flexibility and switching. Humans are normally cocolonized with an average of five commensal *E. coli* strains (1), whereas *E. coli* pathogens are invaders that take over the intestine in the presence of other strains. Thus, metabolic switching might provide a means for several commensal strains to co-inhabit the intestine by dynamic adaptation to host-strain-nutrient variations. The additive cometabolism of substrates by the pathogen might represent more of a slash and burn strategy of competition for resources in which maximum growth potential is achieved by being inflexible and greedy.

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REFERENCES

1. **Apperloo-Renkema, H. Z., B. D. Van der Waaij, and D. Van der Waaij.** 1990. Determination of colonization resistance of the digestive tract by biotyping of Enterobacteriaceae. Epidemiol. Infect. **105:**355–361.
2. **Autieri, S. M., J. J. Lins, M. P. Leatham, D. C. Laux, T. Conway, and P. S. Cohen.** 2007. L-Fucose stimulates utilization of D-ribose by *Escherichia coli* MG1655 Δ*fucAO* and *E. coli* Nissle 1917 Δ*fucAO* mutants in the mouse intestine and in M9 minimal medium. Infect. Immun. **75:**5465–5475.
3. **Blattner, F. R., G. Plunkett III, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao.** 1997. The complete genome sequence of *Escherichia coli* K-12. Science **277:**1453–1474.
4. **Bock, A., and F. C. Neidhardt.** 1966. Properties of a mutant of *Escherichia coli* with a temperature-sensitive fructose-1,6-diphosphate aldolase. J. Bacteriol. **92:**470–476.
5. **Bryant, M. P.** 1974. Nutritional features and ecology of predominant anaerobic bacteria of the intestinal tract. Am. J. Clin. Nutr. **27:**1313–1319.
6. **Chang, D. E., D. J. Smalley, D. L. Tucker, M. P. Leatham, W. E. Norris, S. J. Stevenson, A. B. Anderson, J. E. Grissom, D. C. Laux, P. S. Cohen, and T. Conway.** 2004. Carbon nutrition of *Escherichia coli* in the mouse intestine. Proc. Natl. Acad. Sci. USA **101:**7427–7432.
7. **Conway, T., K. A. Krogfelt, and P. S. Cohen.** 2007. *Escherichia coli* at the intestinal mucosal surface, p. 175–196. *In* K. A. Brogden, F. C. Minion, N. Cornick, T. B. Stanton, Q. Zhang, L. K. Nolan, and M. J. Wannemuehler (ed.), Virulence mechanisms of bacterial pathogens, ed. 4. ASM Press, Washington, DC.

8. **Conway, T., K. A. Krogfelt, and P. S. Cohen.** 29 December 2004, posting date. The life of commensal *Escherichia coli* in the mammalian intestine. Chapter 8.3.1.2. *In* R. Curtis III et al. (ed.), EcoSal—*Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC. http://www.ecosal.org.
9. **Conway, T., and G. K. Schoolnik.** 2003. Microarray expression profiling: capturing a genome-wide portrait of the transcriptome. Mol. Microbiol. **47:**879–889.
10. **Cummings, J. H., and H. N. Englyst.** 1987. Fermentation in the human large intestine and the available substrates. Am. J. Clin. Nutr. **45:**1243–1255.
11. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
12. **Durso, L. M., D. Smith, and R. W. Hutkins.** 2004. Measurements of fitness and competition in commensal *Escherichia coli* and *E. coli* O157:H7 strains. Appl. Environ Microbiol. **70:**6466–6472.
13. **Englyst, K. N., and H. N. Englyst.** 2005. Carbohydrate bioavailability. Br. J. Nutr. **94:**1–11.
14. **Falony, G., A. Vlachou, K. Verbrugghe, and L. De Vuyst.** 2006. Cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose. Appl. Environ Microbiol. **72:**7835–7841.
15. **Flores, S., N. Flores, R. de Anda, A. Gonzalez, A. Escalante, J. C. Sigala, G. Gosset, and F. Bolivar.** 2005. Nutrient-scavenging stress response in an *Escherichia coli* strain lacking the phosphoenolpyruvate:carbohydrate phosphotransferase system, as explored by gene expression profile analysis. J. Mol. Microbiol. Biotechnol. **10:**51–63.
16. **Fraenkel, D. G.** 1968. The accumulation of glucose 6-phosphate from glucose and its effect in an *Escherichia coli* mutant lacking phosphoglucose isomerase and glucose 6-phosphate dehydrogenase. J. Biol. Chem. **243:**6451–6457.
17. **Freter, R.** 1992. Factors affecting the microecology of the gut, p. 111–144. *In* R. Fuller (ed.), Probiotics: the scientific basis. Chapman & Hall, London, United Kingdom.
18. **Freter, R.** 1988. Mechanisms of bacterial colonization of the mucosal surfaces of the gut, p. 45–60. Virulence mechanisms of bacterial pathogens. American Society for Microbiology, Washington, DC.
19. **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.
20. **Henrissat, B., and G. Davies.** 1997. Structural and sequence-based classification of glycoside hydrolases. Curr. Opin. Struct. Biol. **7:**637–644.
21. **Hentges, D. J., J. U. Que, S. W. Casey, and A. J. Stein.** 1984. The influence of streptomycin on colonization in mice. Microecol. Theor. **14:**53–62.
22. **Ho, T. D., and M. K. Waldor.** 2007. Enterohemorrhagic *Escherichia coli* O157:H7 *gal* mutants are sensitive to bacteriophage P1 and defective in intestinal colonization. Infect. Immun. **75:**1661–1666.
23. **Hoskins, L. C., M. Agustines, W. B. McKee, E. T. Boulding, M. Kriaris, and G. Niedermeyer.** 1985. Mucin degradation in human colon ecosystems. Isolation and properties of fecal strains that degrade ABH blood group antigens and oligosaccharides from mucin glycoproteins. J. Clin. Investig. **75:**944–953.
24. **Ihssen, 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.
25. **Irizarry, R. A., B. Hobbs, F. Collin, Y. D. Beazer-Barclay, K. J. Antonellis, U. Scherf, and T. P. Speed.** 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics **4:**249–264.
26. **Jones, S. A., F. Z. Chowdhury, A. J. Fabich, A. Anderson, D. M. Schreiner, A. L. House, S. M. Autieri, M. P. Leatham, J. J. Lins, M. Jorgensen, P. S. Cohen, and T. Conway.** 2007. Respiration of *Escherichia coli* in the mouse intestine. Infect. Immun. **75:**4891–4899.
27. **Kaper, J. B., J. P. Nataro, and H. L. Mobley.** 2004. Pathogenic *Escherichia coli*. Nat Rev. Microbiol. **2:**123–140.
28. **Kovarova-Kovar, K., and T. Egli.** 1998. Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics. Microbiol. Mol. Biol. Rev. **62:**646–666.
29. **Laux, D. C., P. S. Cohen, and T. Conway.** 2005. Role of the mucus layer in bacterial colonization of the intestine, p. 199–212. *In* J. P. Nataro, H. L. T. Mobley, and P. S. Cohen (ed.), Colonization of mucosal surfaces. ASM Press, Washington, DC.
30. **Leatham, M. P., S. J. Stevenson, E. J. Gauger, K. A. Krogfelt, J. J. Lins, T. L. Haddock, S. M. Autieri, T. Conway, and P. S. Cohen.** 2005. 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.
31. **Lendenmann, U., M. Snozzi, and T. Egli.** 2000. Growth kinetics of *Escherichia coli* with galactose and several other sugars in carbon-limited chemostat culture. Can. J. Microbiol. **46:**72–80.
32. **Lendenmann, U., M. Snozzi, and T. Egli.** 1996. Kinetics of the simultaneous utilization of sugar mixtures by *Escherichia coli* in continuous culture. Appl. Environ Microbiol. **62:**1493–1499.
33. **Liu, M., T. Durfee, J. E. Cabrera, K. Zhao, D. J. Jin, and F. R. Blattner.** 2005. Global transcriptional programs reveal a carbon source foraging strategy by *Escherichia coli*. J. Biol. Chem. **280:**15921–15927.
34. **Mayer, C., and W. Boos.** 2005. Hexose/pentose and hexitol/pentitol metabolism. Chapter 3.4.1. *In* R. Curtis III et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 3rd online ed. ASM Press, Washington, DC
35. **Miranda, R. L., T. Conway, M. P. Leatham, D. E. Chang, W. E. Norris, J. H. Allen, S. J. Stevenson, D. C. Laux, and P. S. Cohen.** 2004. Glycolytic and gluconeogenic growth of *Escherichia coli* O157:H7 (EDL933) and *E. coli* K-12 (MG1655) in the mouse intestine. Infect. Immun. **72:**1666–1676.
36. **Møller, A. K., M. P. Leatham, T. Conway, P. J. Nuijten, L. A. de Haan, K. A. Krogfelt, and P. S. Cohen.** 2003. An *Escherichia coli* MG1655 lipopolysaccharide deep-rough core mutant grows and survives in mouse cecal mucus but fails to colonize the mouse large intestine. Infect. Immun. **71:**2142–2152.
37. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119:**736–747.
38. **Peekhaus, N., and T. Conway.** 1998. What's for dinner? Entner-Doudoroff metabolism in *Escherichia coli*. J. Bacteriol. **180:**3495–3502.
39. **Perna, N. T., G. Plunkett III, V. Burland, B. Mau, J. D. Glasner, D. J. Rose, G. F. Mayhew, P. S. Evans, J. Gregor, H. A. Kirkpatrick, G. Posfai, J. Hackett, S. Klink, A. Boutin, Y. Shao, L. Miller, E. J. Grotbeck, N. W. Davis, A. Lim, E. T. Dimalanta, K. D. Potamousis, J. Apodaca, T. S. Anantharaman, J. Lin, G. Yen, D. C. Schwartz, R. A. Welch, and F. R. Blattner.** 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature **409:**529–533.
40. **Perry, M. B., L. MacLean, and D. W. Griffith.** 1986. Structure of the O-chain polysaccharide of the phenol-phase soluble lipopolysaccharide of *Escherichia coli* O:157:H7. Biochem. Cell Biol. **64:**21–28.
41. **Postma, P. W., J. W. Lengeler, and G. R. Jacobson.** 1996 posting date. Phosphoenolpyruvate: carbohydrate phosphotransferase systems, p. 1149–1174. *In* F. C. Neidhardt, R. Curtiss III, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), EcoSal—*Escherichia coli* and *Salmonella:* cellular and molecular biology, 2nd ed. ASM Press, Washington, DC.
42. **Pultz, N. J., L. C. Hoskins, and C. J. Donskey.** 2006. Vancomycin-resistant Enterococci may obtain nutritional support by scavenging carbohydrate fragments generated during mucin degradation by the anaerobic microbiota of the colon. Microb. Drug Resist. **12:**63–67.
43. **Rossi, M., C. Corradini, A. Amaretti, M. Nicolini, A. Pompei, S. Zanoni, and D. Matteuzzi.** 2005. Fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures. Appl. Environ. Microbiol. **71:**6150–6158.
44. **Salyers, A. A., J. K. Palmer, and T. D. Wilkins.** 1978. Degradation of polysaccharides by intestinal bacterial enzymes. Am. J. Clin. Nutr. **31:**S128–S130.
45. **Schnaitman, C. A., and J. D. Klena.** 1993. Genetics of lipopolysaccharide biosynthesis in enteric bacteria. Microbiol. Rev. **57:**655–682.
46. **Schwaiger, H., P. J. Oefner, C. Huber, E. Grill, and G. K. Bonn.** 1994. Capillary zone electrophoresis and micellar electrokinetic chromatography of 4-aminobenzonitrile carbohydrate derivatives. Electrophoresis **15:**941–952.
47. **Stroeher, U. H., L. E. Karageorgos, M. H. Brown, R. Morona, and P. A. Manning.** 1995. A putative pathway for perosamine biosynthesis is the first function encoded within the *rfb* region of *Vibrio cholerae* O1. Gene **166:**33–42.
48. **Tweeddale, H., L. Notley-McRobb, and T. Ferenci.** 1998. Effect of slow growth on metabolism of *Escherichia coli*, as revealed by global metabolite pool ("metabolome") analysis. J. Bacteriol. **180:**5109–5116.
49. **Wolfe, A. J.** 2005. The acetate switch. Microbiol. Mol. Biol. Rev. **69:**12–50.
50. **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.
51. **Zhao, G., J. Liu, X. Liu, M. Chen, H. Zhang, and P. G. Wang.** 2007. Cloning and characterization of GDP-perosamine synthetase (Per) from *Escherichia coli* O157:H7 and synthesis of GDP-perosamine in vitro. Biochem. Biophys. Res. Commun. **363:**525–530.

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