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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▿

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Escherichia coli MG1655 uses several sugars for growth in the mouse intestine. To determine the roles of L-fucose and D-ribose, an _E. coli_ MG1655 Δ_fucAO_ mutant and an _E. coli_ MG1655 Δ_rbsK_ mutant were fed separately to mice along with wild-type _E. coli_ MG1655. The _E. coli_ MG1655 Δ_fucAO_ mutant colonized the intestine at a level 2 orders of magnitude lower than that of the wild type, but the _E. coli_ MG1655 Δ_rbsK_ mutant and the wild type colonized at nearly identical levels. Surprisingly, an _E. coli_ MG1655 Δ_fucAO_ Δ_rbsK_ mutant was eliminated from the intestine by either wild-type _E. coli_ MG1655 or _E. coli_ MG1655 Δ_fucAO_, suggesting that the Δ_fucAO_ mutant switches to ribose in vivo. Indeed, in vitro growth experiments showed that L-fucose stimulated utilization of D-ribose by the _E. coli_ MG1655 Δ_fucAO_ mutant but not by an _E. coli_ MG1655 Δ_fucK_ mutant. Since the Δ_fucK_ mutant cannot convert L-fuculose to L-fuculose-1-phosphate, whereas the Δ_fucAO_ mutant accumulates L-fuculose-1-phosphate, the data suggest that L-fuculose-1-phosphate stimulates growth on ribose both in the intestine and in vitro. An _E. coli_ Nissle 1917 Δ_fucAO_ mutant, derived from a human probiotic commensal strain, acted in a manner identical to that of _E. coli_ MG1655 Δ_fucAO_ in vivo and in vitro. Furthermore, L-fucose at a concentration too low to support growth stimulated the utilization of ribose by the wild-type _E. coli_ strains in vitro. Collectively, the data suggest that L-fuculose-1-phosphate plays a role in the regulation of ribose usage as a carbon source by _E. coli_ MG1655 and _E. coli_ Nissle 1917 in the mouse intestine.

We are interested in identifying the nutrients that *Escherichia coli* uses to successfully colonize the mouse large intestine in the face of competition from an extensive microflora. The large intestine of the mouse consists of the cecum and the colon, each of which contains the mucosa and the luminal contents. The two components of the mucosa are the layer of epithelial cells on the intestinal wall and the mucus layer that covers them. The relatively thick (up to 800 μm) mucus layer consists of mucin, a 2-MDa gel-forming glycoprotein and a large number of smaller glycoproteins, proteins, glycolipids, lipids, and sugars (1, 3, 15, 28, 46, 47, 51, 52). The mucus layer itself is in a dynamic state, constantly being synthesized and secreted by specialized goblet cells and degraded to a large extent by the indigenous intestinal microbes (25, 41). *E. coli* is present both in mucus and luminal contents; however, a large body of experimental evidence shows that growth is rapid in intestinal mucus both in vitro and in vivo but is either poor or completely inhibited in luminal contents (33, 38, 43, 52, 53, 55). It is therefore highly likely that the ability of an *E. coli* strain to grow in intestinal mucus plays a critical role in its ability to colonize the intestine.

The prevalent theory as to how bacteria colonize the mammalian gut has been called the nutrient-niche theory. This theory states that the 500 to 1,000 indigenous species that make up the microflora (39) can coexist in the large intestine as long as each member is able to utilize one or a few limiting nutrients better than all the others and that the rate of growth of any member of the microflora that successfully colonizes is at least equal to the washout rate from the intestine (19, 20, 21). It is the presence of a complete microflora that results in what has been termed colonization resistance, which refers to the ability of the intestinal microflora to resist colonization by an invading bacterium (2, 20, 22, 54). Due to colonization resistance, studies aimed at determining how an invading bacterium colonizes the mouse intestine are difficult, if not impossible, with conventional animals. However, intestinal colonization can be studied with the streptomycin-treated mouse, which has been used extensively to study *E. coli*, *Salmonella enterica* serovar Typhimurium, and *Klebsiella pneumoniae* intestinal colonization (14, 34, 42, 43).

Streptomycin treatment alters the microecology of the cecum, decreasing the populations of facultative anaerobes and some strict anaerobes and creating a niche for organisms such as *E. coli* (23). Nevertheless, populations of the genera *Bacteroides* and *Eubacterium* in cecal contents of streptomycin-treated mice remain largely unchanged (23). Moreover, the overall number of strict anaerobes in the cecal contents of streptomycin-treated and conventional mice is essentially identical (1×10^9 to 2×10^9 CFU/g of contents) (23). Therefore, while the streptomycin-treated mouse model is not perfect, invading microorganisms must compete for nutrients with a large number of strict anaerobes in the intestine, just as they do in conventional animals. This feature makes the streptomycin-treated mouse model the one of choice to identify the nutrients

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E. coli uses to colonize the mouse intestine in the face of competition from an extensive microflora.

Using the streptomycin-treated mouse model, it recently has been shown that *E. coli* MG1655, a human commensal strain for which the genome has been completely sequenced (5), utilizes several sugars simultaneously for growth in the mouse intestine (7). While these results support the nutrient-niche theory of colonization, the mechanisms governing the simultaneous use of sugars are largely unknown. In the present study, we report that while studying the role of L-fucose as a nutrient for *E. coli* MG1655 and *E. coli* Nissle 1917 in the mouse intestine, we found that accumulation of L-fuculose-1-phosphate, an intermediate in the degradation of L-fucose in *E. coli*, stimulates utilization of D-ribose. The present studies therefore raise the possibility that pool sizes of metabolic intermediates play a role in the selection of nutrients used by *E. coli* MG1655 and *E. coli* Nissle 1917 to achieve maximum colonizing ability.

MATERIALS AND METHODS

Bacterial strains. *E. coli* MG1655 Str^r is a spontaneous streptomycin-resistant mutant of the sequenced wild-type *E. coli* MG1655 strain (CGSC 7740) (37). *E. coli* MG1655 Str^r Nal^r is a spontaneous nalidixic acid-resistant mutant of *E. coli* MG1655 Str^r (37). It is referred to in the text as *E. coli* MG1655. The following deletion mutants were constructed from *E. coli* MG1655 Str^r as described below in the section on mutant construction. *E. coli* MG1655 Str^r Δ*fucAO*::*kan* is both streptomycin resistant and kanamycin resistant and has a 1,542-bp deletion within the 1,823-bp *fucA* and *fucO* genes. It is unable to convert fuculose-1-phosphate to dihydroxyacetone phosphate and lactaldehyde and lacks both L-fuculose phosphate aldolase and propanediol oxidoreductase. It is referred to in the text as *E. coli* MG1655 Δ*fucAO*. *E. coli* MG1655 Str^r Δ*rbsK*::*cam* has a 759-bp deletion of the 929-bp *rbsK* gene (7). It is unable to convert ribose to ribose-5-phosphate, lacks ribokinase, and is both streptomycin and chloramphenicol resistant. It is referred to in the text as *E. coli* MG1655 Δ*rbsK*. *E. coli* MG1655 Str^r Δ*fucAO* Δ*rbsK*::*cam* has the identical 1,542-bp deletion in the *fucA* and *fucO* genes as described above but is missing the kanamycin cassette. In addition, it has a 759-bp deletion in the *rbsK* gene as described above. It is both streptomycin resistant and chloramphenicol resistant and is referred to in the text as *E. coli* MG1655 Δ*fucAO* Δ*rbsK*. *E. coli* MG1655 Str^r Δ*fucK*::*kan* has a 993-bp deletion of the 1,448-bp *fucK* gene. It is unable to convert fuculose to fuculose-1-phosphate, lacks L-fuculokinase, and is both streptomycin and kanamycin resistant. Wild-type *E. coli* Nissle 1917 was obtained from Dean Hamer of the National Cancer Institute. *E. coli* Nissle 1917 Str^r is a spontaneous streptomycin-resistant mutant of the wild-type strain. *E. coli* Nissle 1917 Str^r Nal^r is a spontaneous nalidixic acid-resistant mutant of *E. coli* Nissle 1917 Str^r and is referred to in the text as *E. coli* Nissle 1917. The following deletion mutants were constructed from *E. coli* Nissle 1917 Str^r as described below (see the section on mutant construction): *E. coli* Nissle 1917 Str^r Δ*fucAO*::*kan*, *E. coli* Nissle 1917 Str^r Δ*rbsK*::*kan*, *E. coli* Nissle 1917 Str^r Δ*fucAO* Δ*rbsK*::*cam*, and *E. coli* Nissle 1917 Str^r Δ*fucK*::*cam*. The mutants contain the same deletions as their *E. coli* MG1655 counterparts and are referred to in the text as *E. coli* Nissle 1917 Δ*fucAO*, *E. coli* Nissle 1917 Δ*rbsK*, *E. coli* Nissle 1917 Δ*fucAO* Δ*rbsK*, and *E. coli* Nissle 1917 Δ*fucK*, respectively.

Media and growth conditions. Luria broth (LB) was made as described by Revel (48). Luria agar is LB containing 12 g of Bacto agar (Difco) per liter. MacConkey agar (Difco) was prepared according to the manufacturer's instructions. For testing the ability of *E. coli* strains to utilize D-ribose or L-fucose, overnight cultures grown in LB were washed twice in M9 minimal medium (no carbon source), 10 μl of the washed cultures was transferred to 10 ml of M9 minimal medium (35) containing reagent-grade glycerol (0.4%, wt/wt) as the sole carbon and energy source, and cultures were incubated at 37°C with shaking in 125-ml tissue culture bottles. Growth and the lack of growth were assessed visually. For testing the ability of L-fucose to induce the utilization of D-ribose in *E. coli* MG1655 and in *E. coli* Nissle 1917 Δ*fucAO* and Δ*fucK* mutants, the mutants were grown twice in M9 minimal medium containing glycerol (0.2%, wt/wt) as described above and then were washed and resuspended at an A_{600} of 0.1 in three separate 20-ml volumes of M9 minimal medium containing either 0.05% fucose, 0.15% ribose, or 0.05% fucose and 0.15% ribose. The cultures were incubated at 37°C with shaking in 125-ml tissue culture bottles. Growth was monitored spectrophotometrically (A_{600}) using a Pharmacia Biotech Ultrospec 2000 UV/visible spectrophotometer.

Mutant construction. Mutant *E. coli* strains were created by deletion mutagenesis using either a chloramphenicol cassette or a kanamycin cassette as described by Datsenko and Wanner (12). Primers used to construct deletion mutants were designed according to the *E. coli* MG1655 genome sequence (5). DNA procedures were as described previously (37). All constructs were verified by PCR and sequencing. The primers used to construct the Δ*fucAO* deletions were the following (uppercase letters, MG1655 DNA; lowercase letters, kanamycin cassette DNA): primer 1, 5′-CGCAAATGTGGCGGAATACCGACATCACGGTTGAGAGCAAACAgtgtaggctggagctgcttcg-3′; primer 2, 5′-CGTCAGTGTACGTTATCAGGATGGGATGCTGATTACGCCTACAGGCAtatgaatatcctccttag-3′. The primers used to construct the Δ*rbsK* deletions were the following (uppercase letters, MG1655 DNA; lowercase letters, chloramphenicol or kanamycin cassette DNA): primer 1, 5′-TGGCAGCATTAATGCTGACCACATTCTTAATCTTCAATCTTTTCCTACTCtgtaggctggagctgcttcg-3′; primer 2, 5′-GGTACGGAAGGTTGTGCGCCTTTACGTGTTACGGCAATCGCAGCGGCAGGCAtatgaatatcctccttag-3′. The primers used to construct the Δ*fucK* deletions were the following (uppercase letters, MG1655 DNA; lowercase letters, chloramphenicol or kanamycin cassette DNA): primer 1, 5′-CTGTGGCGCGACCAATGTCAGGGCCATCGCGGTTAATCGGCAGGGgtgtaggctggagctgcttcg-3′; primer 2, 5′-GTACGACTTAACAGCGAAGTATCAACCTGGGCGCTGCGAACCATCAtatgaatatcctccttag-3′.

Sequencing. DNA sequencing was done at the URI Genomics and Sequencing Center, University of Rhode Island, Kingston, using the CEQ8000 genetic analysis system (Beckman Coulter, Fullerton, CA). The dye terminator cycle sequencing quick start kit (Beckman Coulter) was used in the sequencing reactions. The primers used to amplify PCR products for sequencing to determine the precise location of the deletions for *E. coli* MG1655 Δ*fucK* and *E. coli* Nissle 1917 Δ*fucK* were 5′-TATGCACAACGTTGAAGACACC-3′ and 5′-CCACAATGTGTTGCGACTTCCTC-3′. The same primers were used for sequencing. For *E. coli* MG1655 Δ*fucAO* and *E. coli* Nissle 1917 Δ*fucAO* PCR amplification, the primers were the following: 5′-GCTTACAAACCGATTTGCATATC-3′ and 5′-GTGGGTAATTAAACGGCTAATTC-3′; for sequencing, the primers were 5′-AACAGTACTGCGATGAGTGGCAG-3′ and 5′-GCGAAGTGATCTTCCGTCACAGGT-3′. For *E. coli* MG1655 Δ*rbsK* PCR amplification, the primers were 5′-GGTTGTATGACCTGATGGTGAC-3′ and 5′-GAGAAACTG TTGAGGTAGAAACG-3′. The same primers were used for sequencing. For *E. coli* Nissle 1917 Δ*rbsK* PCR amplification, the primers were 5′-CGTTGTATGACCTGATGGTGAC-3′ and 5′-GAGAAACT GTTGAGGTAGAAACG-3′. The same primers were used for sequencing.

Mouse colonization experiments. The method used to compare the large intestine-colonizing abilities of *E. coli* strains in mice has been described previously (52, 53, 55). 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 (36). Following 18 h of starvation for food and water, the mice were fed 1 ml of 20% (wt/vol) sucrose containing LB-grown *E. coli* strains, as described in the Results section. After ingesting 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 were placed in clean cages daily. Fecal samples (no older than 24 h) were homogenized in 1% Bacto tryptone, diluted in the same medium, and plated on MacConkey agar plates with appropriate antibiotics. Plates contained streptomycin sulfate (100 μg/ml) and nalidixic acid (50 μg/ml), streptomycin sulfate (100 μg/ml) and kanamycin sulfate (40 μg/ml), or streptomycin sulfate (100 μg/ml) and chloramphenicol (30 μg/ml). Antibiotics were purchased from Sigma-Aldrich (St. Louis, MO). All plates were incubated for 18 to 24 h at 37°C prior to counting. Each colonization experiment was performed at least twice, with essentially identical results. Pooled data from at least two independent experiments are presented in the figures.

RESULTS

Sequencing and growth of mutants in vitro. All mutants used in this study were sequenced to be sure that the deletions were in the expected places in the chromosome (see Materials and Methods). In addition, each mutant was tested for growth in M9 minimal medium containing 0.4% (wt/wt) glycerol, fucose, or ribose as the sole carbon and energy source. All mutants grew normally with glycerol as the sole carbon and energy source. Furthermore, all mutants with a Δ*fucAO* deletion or a

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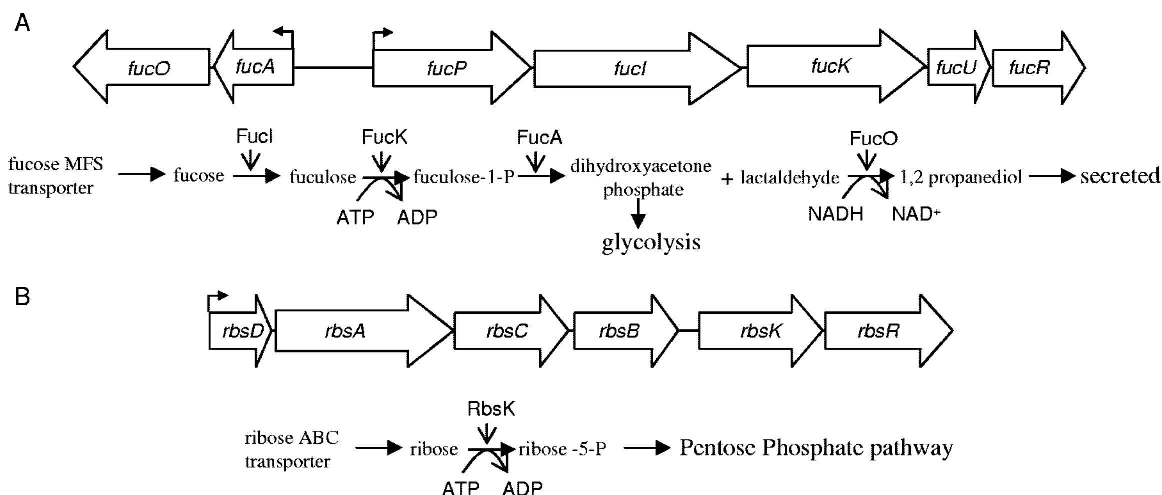

FIG. 1. Fucose operons and degradation pathway (A) and the ribose operon and degradation pathway (B). Arrows above genes indicate promoters and the direction of transcription. MFS, major facilitator superfamily; ABC, ATP-binding cassette.

Δ*fucK* deletion failed to grow with fucose as the sole carbon and energy source, and those with a Δ*rbsK* deletion failed to grow with ribose as the sole carbon and energy source. Moreover, Δ*fucAO* Δ*rbsK* double mutants failed to grow on a mixture of fucose and ribose.

Mouse intestinal colonization of *E. coli* MG1655 Δ*fucAO* and Δ*rbsK* mutants. Unlike its wild-type parent, *E. coli* MG1655 Δ*fucAO* cannot convert fuculose-1-phosphate to dihydroxyacetone phosphate and lactaldehyde (Fig. 1A) and therefore is unable to use fucose as a carbon and energy source. Similarly, *E. coli* MG1655 Δ*rbsK* cannot phosphorylate ribose to ribose-5-phosphate and therefore is unable to utilize ribose as a carbon and energy source (Fig. 1B). When mice were fed 10^5 CFU each of wild-type *E. coli* MG1655 and *E. coli* MG1655 Δ*fucAO*, the wild-type *E. coli* MG1655 grew to a level of about 10^8 CFU/g of feces within 1 day after feeding and then within a few days stabilized at a level of about 10^7 CFU/g of feces, whereas *E. coli* MG1655 Δ*fucAO* initially grew to a level of only about 10^7 CFU/g of feces and within a few days stabilized at a level of about 10^5 CFU/g of feces (Fig. 2A). When mice were fed 10^5 CFU each of wild-type *E. coli* MG1655 and *E. coli* MG1655 Δ*rbsK*, both grew at the same rate in the intestine to about 10^8 CFU/g of feces, and only thereafter did the *E. coli* MG1655 wild type, which stabilized at a level of about 10^7 CFU/g of feces, develop a very slight advantage, at most fourfold, over *E. coli* MG1655 Δ*rbsK* (Fig. 2B). Therefore, *E. coli* MG1655 appeared to use fucose during the initiation stage of the colonization process but appeared to stop using it during maintenance. In contrast, ribose did not appear to be used to any great extent during either stage of colonization.

***E. coli* MG1655 Δ*fucAO* appears to switch to ribose in the intestine.** Since *E. coli* MG1655 Δ*rbsK* was nearly as good a colonizer as wild-type *E. coli* MG1655, it was expected that the colonizing ability of an *E. coli* MG1655 Δ*fucAO* Δ*rbsK* double mutant would closely mimic the colonizing ability of *E. coli* MG1655 Δ*fucAO*. Surprisingly, when mice were fed 10^5 CFU each of the wild-type *E. coli* MG1655 and *E. coli* MG1655 Δ*fucAO* Δ*rbsK*, the double mutant proved to be a far worse colonizer than expected, essentially being eliminated by day 15 after feeding (Fig. 2C). These data suggested the possibility that the *E. coli* MG1655 Δ*fucAO* mutant switched to ribose in the intestine. If true, the *E. coli* MG1655 Δ*fucAO* Δ*rbsK* double mutant should be a far worse colonizer than the *E. coli* MG1655 Δ*fucAO* mutant, despite differing only in the ability to utilize ribose for growth. Indeed, the *E. coli* MG1655 Δ*fucAO* Δ*rbsK* double mutant was rapidly eliminated in competition with *E. coli* MG1655 Δ*fucAO* (Fig. 2D), suggesting that the *E. coli* MG1655 Δ*fucAO* mutant, in contrast to the wild-type *E. coli* MG1655, utilizes ribose for growth in the intestine.

When mice are fed high numbers (10^{10} CFU/mouse) of a wild-type *E. coli* strain (resistant to streptomycin) and low numbers (10^5 CFU/mouse) of the same wild-type strain (e.g., resistant to streptomycin and nalidixic acid), they maintain the initial ratio of their input values (52), as would be expected of two strains that use all nutrients equally well. It then would be expected that if wild-type *E. coli* MG1655 was fed to mice in high numbers (10^{10} CFU/mouse) and the *E. coli* MG1655 Δ*fucAO* mutant was fed to the same mice in low numbers (10^5 CFU/mouse) and the only difference between the two strains was their ability to utilize fucose for growth, the Δ*fucAO* mutant would stabilize in numbers 5 orders of magnitude lower than those for both strains being fed to mice in equal numbers, i.e., a total of about 7 orders of magnitude. On the other hand, if the *E. coli* MG1655 Δ*fucAO* mutant was using ribose and the wild-type *E. coli* MG1655 was not, the *E. coli* MG1655 Δ*fucAO* mutant could conceivably grow from low numbers to higher numbers in the presence of high numbers of the wild-type strain and could colonize at the level observed when both strains were fed to the mice in low numbers, i.e., only 2 orders of magnitude lower than the level of the wild type (Fig. 1A). This is precisely what happened, i.e., when mice were fed 10^{10} CFU of the wild-type *E. coli* MG1655 and 10^5 CFU of the *E. coli* MG1655 Δ*fucAO* mutant, the mutant grew from a level of 5 orders of magnitude lower than that of its parent to only 2 orders of magnitude lower within a few days (Fig. 2E).

The switch to ribose appears to require fuculose-1-phosphate. Because the *E. coli* MG1655 Δ*fucAO* mutant cannot

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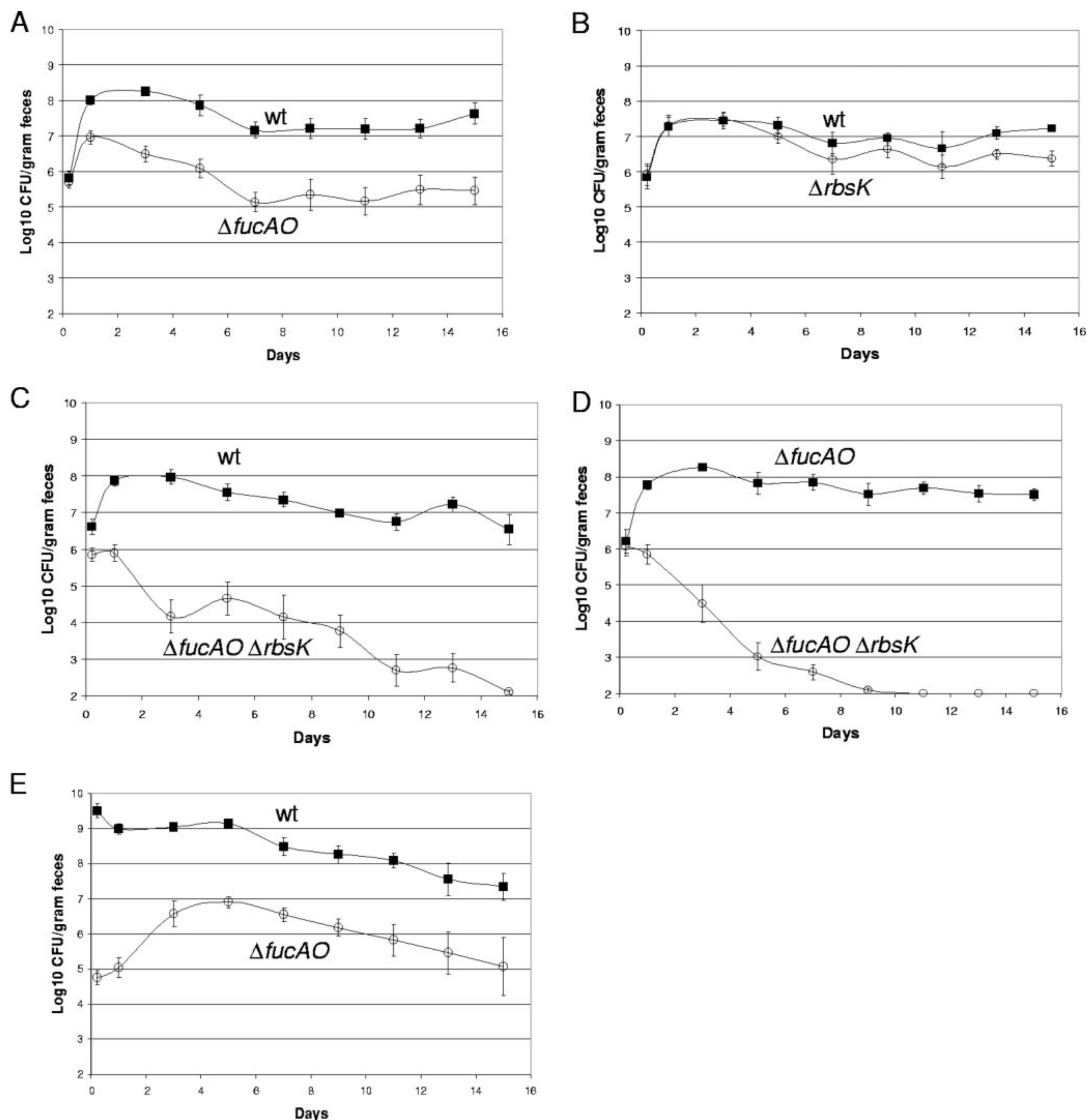

FIG. 2. Colonization of the mouse intestine by *E. coli* MG1655 Δ*fucAO*, *E. coli* MG1655 Δ*rbsK*, and *E. coli* MG1655 Δ*fucAO* Δ*rbsK*. Sets of three mice were fed 10^5 CFU of *E. coli* MG1655 (■) and 10^5 CFU of MG1655 Δ*fucAO* (○) (A); 10^5 CFU of *E. coli* MG1655 (■) and 10^5 CFU of *E. coli* MG1655 Δ*rbsK* (○) (B); 10^5 CFU of *E. coli* MG1655 (■) and 10^5 CFU of *E. coli* MG1655 Δ*fucAO* Δ*rbsK* (○) (C); 10^5 CFU of *E. coli* MG1655 Δ*fucAO* (■) and 10^5 CFU of *E. coli* MG1655 Δ*fucAO* Δ*rbsK* (○) (D); and 10^{10} CFU of *E. coli* MG1655 (■) and 10^5 CFU of *E. coli* MG1655 Δ*fucAO* (○) (E). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars representing standard errors of the $\log_{10}$ means of CFU per gram of feces for six mice are presented for each time point except for panels A and B, for which data from 12 mice are presented. Each colonization curve has the specific strain genotype immediately above or below it. wt, wild-type *E. coli* MG1655.

convert fuculose-1-phosphate to dihydroxyacetone phosphate and lactaldehyde (8) and because fuculose-1-phosphate is required for induction of the *fucPIKUR* operon (4), *E. coli* MG1655 Δ*fucAO* most likely accumulates fuculose-1-phosphate in the presence of fucose. Since the *E. coli* MG1655 Δ*fucAO* mutant appeared to be using ribose for growth in the intestine, the question was whether the switch to ribose requires the presence of fuculose-1-phosphate. An *E. coli*

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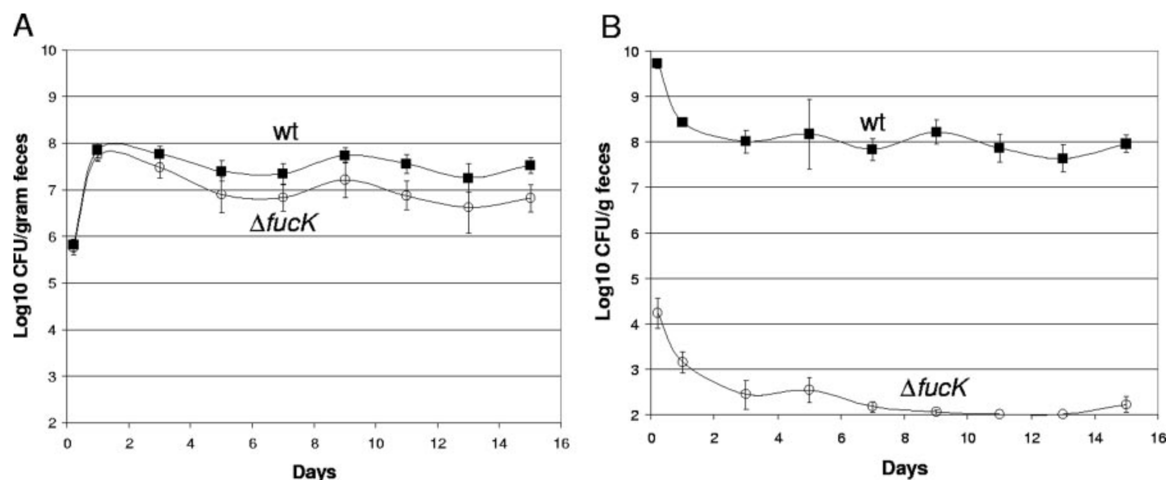

FIG. 3. Colonization of the mouse intestine by *E. coli* MG1655 Δ*fucK*. Sets of three mice were fed 10^5 CFU of *E. coli* MG1655 (■) and 10^5 CFU of MG1655 Δ*fucK* (○) (A) or 10^{10} CFU of *E. coli* MG1655 (■) and 10^5 CFU of *E. coli* MG1655 Δ*fucK* (○) (B). At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Bars representing standard errors of the $\log_{10}$ means of CFU per gram of feces for 12 mice are presented for each time point in panel A and for 6 mice in panel B. Each colonization curve has the specific strain genotype immediately above or below it. wt, wild-type *E. coli* MG1655.

MG1655 Δ*fucK* mutant, which is defective in the phosphorylation of fuculose to fuculose-1-phosphate (Fig. 1A), was employed to address this question. When mice were fed 10^5 CFU each of wild-type *E. coli* MG1655 and *E. coli* MG1655 Δ*fucK*, the mutant colonized at most fivefold below the level of its wild-type parent (Fig. 3A). When the *E. coli* MG1655 Δ*fucK* mutant was fed to mice at 10^5 CFU/mouse and wild-type *E. coli* MG1655 was simultaneously fed at 10^{10} CFU/mouse, the *E. coli* MG1655 Δ*fucK* mutant was eliminated (Fig. 3B). Therefore, the *E. coli* MG1655 Δ*fucK* mutant does not use ribose, and neither does it switch to any other nutrient in the intestine to allow it to grow up in the presence of high numbers of wild-type *E. coli* MG1655. Moreover, since the only difference between the *E. coli* MG1655 Δ*fucK* mutant and the *E. coli* MG1655 Δ*fucAO* mutant is the inability of the *E. coli* MG1655 Δ*fucK* mutant to accumulate fuculose-1-phosphate, i.e., neither mutant is able to make dihydroxyacetone phosphate and lactaldehyde (Fig. 1A), it would appear that the switch of *E. coli* MG1655 Δ*fucAO* to ribose in the intestine requires the accumulation of fuculose-1-phosphate.

Fucose stimulates the utilization of ribose for growth by *E. coli* MG1655 Δ*fucAO* in vitro but not for growth by *E. coli* MG1655 Δ*fucK*. Since it appeared that fucose stimulates utilization of ribose by *E. coli* MG1655 Δ*fucAO* in the intestine, growth experiments were performed to determine whether fucose could stimulate utilization of ribose by *E. coli* MG1655 Δ*fucAO* in vitro. *E. coli* MG1655 Δ*fucAO* and *E. coli* MG1655 Δ*fucK* were grown in M9 minimal medium in the presence of 0.05% fucose, 0.15% ribose, and a mixture of 0.05% fucose and 0.15% ribose. As expected, neither strain grew with fucose as the sole carbon and energy source (Fig. 4). However, fucose stimulated more rapid growth of *E. coli* MG1655 Δ*fucAO* on ribose (Fig. 4A) but had no effect on the rate of growth of *E. coli* MG1655 Δ*fucK* on ribose (Fig. 4B). That the stimulation of ribose utilization required a functional *rbsK* gene was shown by the fact that the *E. coli* MG1655 Δ*fucAO* Δ*rbsK* mutant failed to grow on ribose in the presence or absence of fucose (data not shown). Collectively, these in vitro data support the idea that it is accumulation of fuculose-1-phosphate that stimulates growth of *E. coli* MG1655 Δ*fucAO* on ribose in the mouse intestine.

Fucose has no effect on utilization of *N*-acetylglucosamine, but it inhibits the utilization of galactose and mannose for growth by *E. coli* MG1655 Δ*fucAO* in vitro. Both *E. coli* MG1655 Δ*fucK* and *E. coli* MG1655 Δ*fucAO* are unable to use fucose for growth, but *E. coli* MG1655 Δ*fucAO* switches to ribose in the intestine, whereas the switch to ribose is not made by *E. coli* MG1655 Δ*fucK.* Why then is *E. coli* MG1655 Δ*fucK* a better colonizer than *E. coli* MG1655 Δ*fucAO* when each is in competition with wild-type *E. coli* MG1655 (compare Fig. 2A to 3A)? One possibility was that while accumulation of fuculose-1-phosphate in *E. coli* MG1655 Δ*fucAO* stimulates utilization of ribose, it might simultaneously inhibit utilization of other sugars. Growth experiments analogous to those described for the effect of fucose on ribose utilization also were performed to determine the effect of fucose on utilization of *N*-acetylglucosamine, galactose, and mannose for growth by *E. coli* MG1655 Δ*fucAO* and *E. coli* MG1655 Δ*fucK* in vitro. Indeed, although 0.05% fucose had no effect on the utilization of 0.15% *N*-acetylglucosamine by *E. coli* MG1655 Δ*fucAO* in vitro (Fig. 5A), it greatly reduced the utilization of both galactose and mannose (Fig. 5B and C). In contrast, utilization of *N*-acetylglucosamine, galactose, and mannose was unaltered by fucose in the *E. coli* MG1655 Δ*fucK* mutant (data not shown). It therefore seems likely that *E. coli* MG1655 Δ*fucAO* is a worse colonizer than *E. coli* MG1655 Δ*fucK*, despite using ribose more efficiently, because accumulation of fuculose-1-phosphate inhibits its ability to compete for other sugars that are normally used for growth in the intestine.

Fucose stimulates ribose utilization for growth by wild-type *E. coli* MG1655 in vitro. In vitro growth experiments also were performed to determine whether fucose could stimulate utilization of ribose by wild-type *E. coli* MG1655 in vitro. In this case, it was necessary to use a concentration of fucose that

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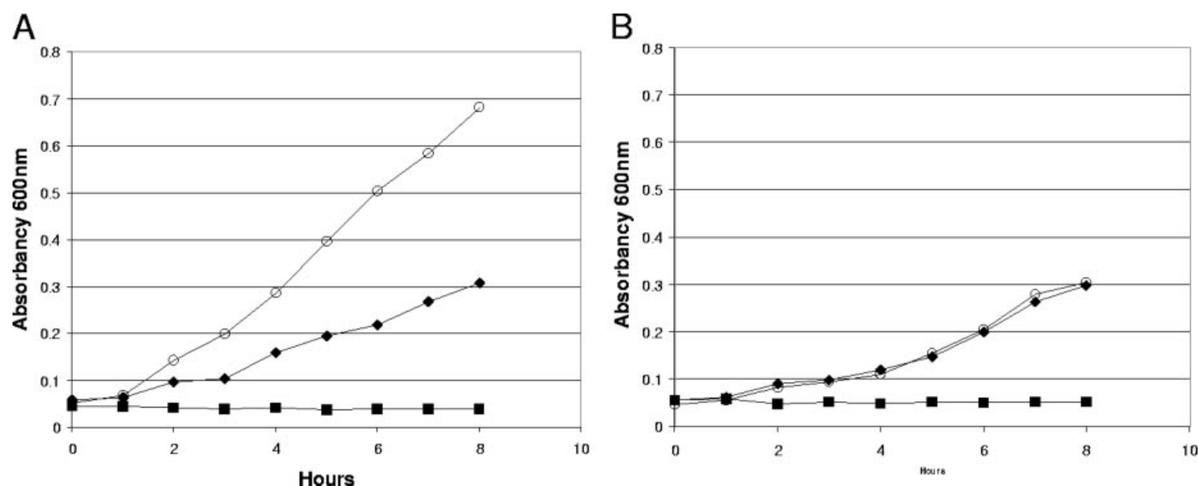

FIG. 4. Growth of *E. coli* MG1655 Δ*fucAO* (A) and *E. coli* MG1655 Δ*fucK* (B) in M9 minimal medium in the presence of 0.05% (wt/wt) fucose (■), 0.15% (wt/wt) ribose (◆), or a mixture of 0.05% (wt/wt) fucose and 0.15% (wt/wt) ribose (○). *E. coli* MG1655 Δ*fucAO* and *E. coli* MG1655 Δ*fucK* were grown in M9 minimal medium containing glycerol (0.2%, wt/wt), washed, and resuspended in M9 minimal medium containing the appropriate sugars (see Materials and Methods). Incubation was at 37°C with aeration. A_{600} readings at the indicated times are presented. Growth experiments were performed at least three times. The results of typical experiments are shown.

would not allow *E. coli* MG1655 to grow but might still stimulate ribose utilization. Therefore, *E. coli* MG1655 was grown in the presence of 0.005% fucose, 0.15% ribose, or a mixture of 0.005% fucose and 0.15% ribose. Wild-type *E. coli* MG1655 did not grow in the presence of 0.005% fucose, but 0.005% fucose did stimulate the utilization of ribose by wild-type *E. coli* MG1655 (Fig. 6). It therefore appears that stimulation of ribose utilization by fucose also can occur in wild-type *E. coli* MG1655 at fucose concentrations too low to allow growth, suggesting the possibility that under the right conditions, fucose could signal wild-type *E. coli* MG1655 to utilize ribose in the intestine.

***E. coli* Nissle 1917 Δ*fucAO* also switches to ribose in the intestine.** Experiments were conducted to determine whether the switch to ribose in the intestine by *E. coli* MG1655 Δ*fucAO* was an isolated case or whether other *E. coli* strains carry out the switch. *E. coli* Nissle 1917 is a commensal strain that has been used successfully as a probiotic agent to treat gastrointestinal infections in humans since the early 1920s (50). Like *E. coli* MG1655 Δ*fucAO*, an *E. coli* Nissle 1917 Δ*fucAO* mutant colonized at a level about 2 orders of magnitude less than that of its wild-type parent when 10^5 CFU of each strain was fed to mice (Table 1). Also like *E. coli* MG1655 Δ*rbsK*, an *E. coli* Nissle 1917 Δ*rbsK* mutant colonized at a level of only about fourfold lower than that of its wild-type parent when 10^5 CFU of each strain was fed to mice (Table 1). In addition, an *E. coli* Nissle 1917 Δ*fucAO* Δ*rbsK* mutant colonized at a level of about 5 orders of magnitude lower than that of its wild-type parent when 10^5 CFU of each strain was fed to mice, i.e., greater than 2 orders of magnitude lower than expected (Table 1). Furthermore, when mice were fed 10^{10} CFU of wild-type *E. coli* Nissle 1917 and 10^5 CFU of the *E. coli* Nissle 1917 Δ*fucAO* mutant, the mutant grew from a level of 5 orders of magnitude lower than that of its parent to between 1.5 and 2 orders of magnitude lower within a few days (Table 1). Therefore, like *E. coli* MG1655 Δ*fucAO*, the *E. coli* Nissle 1917 Δ*fucAO* mutant appears to use ribose for growth in the intestine.

Mice also were fed 10^5 CFU each of wild-type *E. coli* Nissle 1917 and an *E. coli* Nissle 1917 Δ*fucK* mutant. The *E. coli* Nissle 1917 Δ*fucK* mutant colonized at a level of between 1 and 2 orders of magnitude lower than that of the wild-type strain (Table 1). In addition, when mice were fed 10^{10} CFU of wild-type *E. coli* Nissle 1917 and 10^5 CFU of *E. coli* Nissle 1917 Δ*fucK*, the *E. coli* Nissle 1917 Δ*fucK* mutant was essentially eliminated (Table 1). Collectively, these data suggest that the *E. coli* Nissle 1917 Δ*fucK* mutant does not switch to ribose in the intestine and that, like the *E. coli* MG1655 Δ*fucAO* mutant, the *E. coli* Nissle 1917 Δ*fucAO* mutant switches to ribose for growth in the intestine, mediated by the accumulation of fuculose-1-phosphate.

Fucose stimulates the utilization of ribose by wild-type *E. coli* Nissle 1917 and by *E. coli* Nissle 1917 Δ*fucAO* for growth in vitro. Growth experiments were performed to determine whether fucose could stimulate utilization of ribose in the *E. coli* Nissle 1917 Δ*fucAO* mutant in vitro. The *E. coli* Nissle 1917 Δ*fucAO* mutant and the *E. coli* Nissle 1917 Δ*fucK* mutant were grown in vitro in the presence of 0.05% fucose, 0.15% ribose, and 0.05% fucose plus 0.15% ribose. As expected, neither strain grew on fucose as the sole carbon and energy source. However, fucose was able to induce more rapid growth of *E. coli* Nissle 1917 Δ*fucAO* on ribose (Fig. 7A). Fucose did not induce more rapid growth of the *E. coli* Nissle 1917 Δ*fucK* mutant on ribose (Fig. 7B). Moreover, fucose had no effect on *N*-acetylglucosamine utilization but inhibited both mannose and galactose utilization in the *E. coli* Nissle 1917 Δ*fucAO* mutant (data not shown). Fucose had no effect on *N*-acetylglucosamine, galactose, or mannose utilization by *E. coli* Nissle 1917 Δ*fucK* (data not shown). It therefore appears that as in the case of *E. coli* MG1655, accumulation of fuculose-1-phosphate induces more rapid growth of the commensal *E. coli* Nissle 1917 Δ*fucAO* mutant on ribose in vitro, has no effect on *N*-acetylglucosamine utilization, and inhibits both galactose and mannose utilization. Finally, wild-type *E. coli* Nissle 1917 was grown in the presence of 0.005% fucose, 0.15% ribose, or

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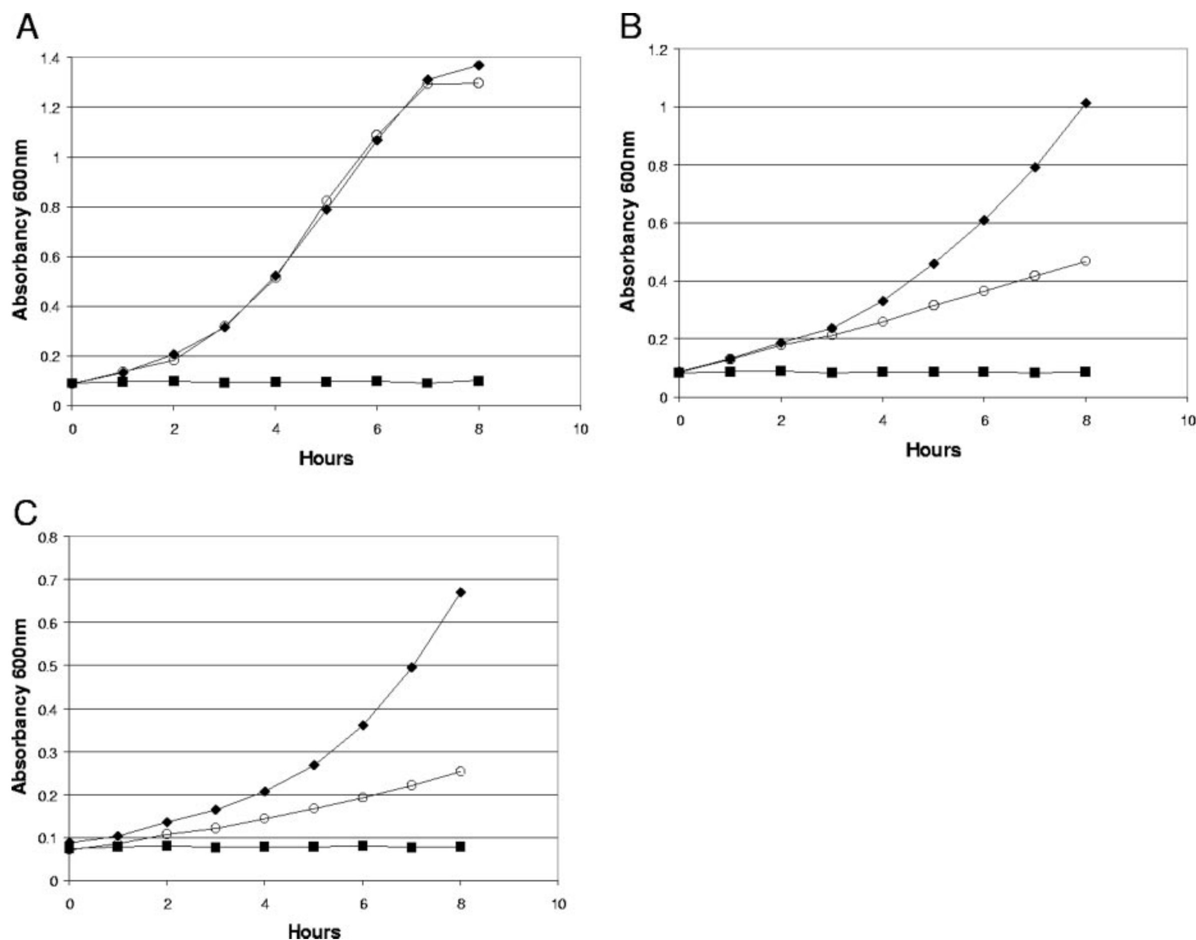

FIG. 5. Growth of *E. coli* MG1655 Δ*fucAO* in M9 minimal medium in the presence of 0.05% (wt/wt) fucose (■), 0.15% (wt/wt) *N*-acetylglucosamine (◆), or a mixture of 0.05% (wt/wt) fucose and 0.15% (wt/wt) *N*-acetylglucosamine (○) (A); 0.05% (wt/wt) fucose (■), 0.15% (wt/wt) mannose (◆), or a mixture of 0.05% (wt/wt) fucose and 0.15% (wt/wt) mannose (○) (B); and 0.05% (wt/wt) fucose (■), 0.15% (wt/wt) galactose (◆), or a mixture of 0.05% (wt/wt) fucose and 0.15% (wt/wt) galactose (○) (C). *E. coli* MG1655 Δ*fucAO* was grown in M9 minimal medium containing glycerol (0.2%, wt/wt), washed, and resuspended in M9 minimal medium containing the appropriate sugars (see Materials and Methods). Incubation was at 37°C with aeration. A_{600} readings at the indicated times are presented. Growth experiments were performed at least three times. The results of typical experiments are shown.

a mixture of 0.005% fucose and 0.15% ribose. Again, like *E. coli* MG1655, the wild-type *E. coli* Nissle 1917 did not grow in the presence of 0.005% fucose, but 0.005% fucose did stimulate its use of ribose (Fig. 7C).

DISCUSSION

It is becoming increasingly clear that in the face of competition from a complex microflora, *E. coli* simultaneously uses several, presumably limiting, sugars for growth in the mouse intestine, including D-gluconate, *N*-acetyl-D-glucosamine, D-glucuronate, and sialic acid (7, 10, 30, 31). The simultaneous use of several sugars for growth is not unprecedented. In fact, *E. coli* has been shown to utilize D-glucose, D-galactose, D-maltose, D-ribose, L-arabinose, and D-fructose simultaneously in a chemostat under carbon-limiting conditions (32). It also appears that *E. coli* is capable of preparing itself for the appearance of alternative carbon sources when growing under glucose-limiting conditions or when utilizing acetate as the sole carbon source (18, 44). Thus, DNA microarray analysis has shown that when *E. coli* was grown in a chemostat under glucose-limiting conditions, genes associated with the maltose and galactose operons were up-regulated relative to batch cultures grown under conditions in which glucose was not limited (18). Similarly, with acetate as the sole carbon source, genes encoding transporters for galactose, ribose, and *N*-acetylglucosamine were up-regulated relative to growth with glucose as the sole carbon source (44). Moreover, using Biolog AN MicroPlate respiration analysis, it has recently been shown that when *E. coli* MG1655 was grown in a chemostat under glucose-limiting conditions, despite the absence of inducers a wide variety of catabolic functions were derepressed (26).

There are two divergently transcribed operons for fucose utilization in *E. coli* (Fig. 1A), the *fucPIKUR* operon, which takes fucose to fuculose-1-phosphate, and the *fucAO* operon, which takes fuculose-1-phosphate to dihydroxyacetone phosphate and lactaldehyde (8). Fuculose-1-phosphate is the effector of induction of both fucose operons through the FucR activator protein (4, 8, 9). In the present study, evidence is

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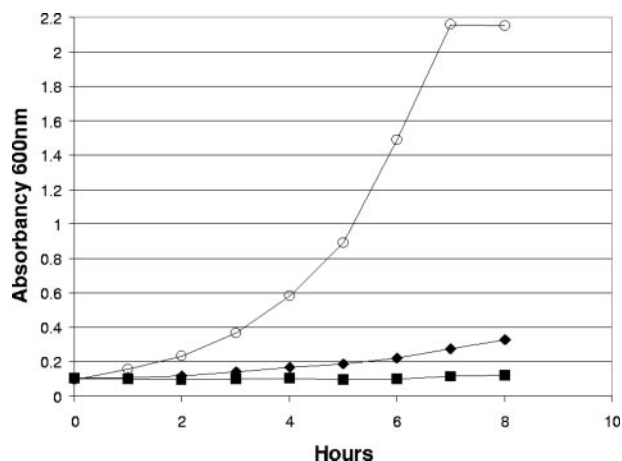

FIG. 6. Growth of wild-type *E. coli* MG1655 in M9 minimal medium in the presence of 0.005% (wt/wt) fucose (■), 0.15% (wt/wt) ribose (◆), or a mixture of 0.005% (wt/wt) fucose and 0.15% (wt/wt) ribose (○). Wild-type *E. coli* MG1655 was grown in M9 minimal medium containing glycerol (0.2%, wt/wt), washed, and resuspended in M9 minimal medium containing the appropriate sugars (see Materials and Methods). Incubation was at 37°C with aeration. A_{600} readings at the indicated times are presented. Growth experiments were performed at least three times. The results of typical experiments are shown.

presented suggesting that accumulation of fuculose-1-phosphate through induction of the *fucPIKUR* operon in two commensal strains, *E. coli* MG1655 Δ*fucAO* and *E. coli* Nissle 1917 Δ*fucAO*, stimulates growth on ribose both in the intestine and in vitro. However, in a *fucAO* mutant, in addition to accumulation of fuculose-1-phophate, it would be expected that the fuculose transporter (FucP) encoded by *fucP*, the FucR activator protein encoded by *fucR*, and the L-fucose mutarotase encoded by *fucU* also would accumulate. It recently has been reported (49) that the L-fucose mutarotase can convert β-D-pyranoribose, the form transported into the cell, to α-D-ribofuranose (49). α-D-Ribofuranose is required for ribose catabolism (40). It also has recently been reported that D-fructose possibly can enter an *E. coli fucA* mutant via FucP (29). If ribose were to enter Δ*fucAO* cells via FucP, it then could induce the ribose operon. In this case and in the case of the mutarotase, the fuculose-1-phosphate would be necessary only for inducing the *fucPIKUR* operon. Whether the L-fucose permease and/or the L-fucose mutarotase is involved in stimulation of ribose utilization is presently under investigation.

At the present time, it is not clear how or if fuculose-1-phosphate is involved either by itself or as an effector in combination with a regulatory protein in inducing the *rbsDABCKR* operon. It is possible that accumulated fuculose-1-phosphate acts as an effector of FucR to directly activate or release repression of transcription of the *rbsDABCKR* operon. In *Bacteroides thetaiotaomicron*, L-fucose has been implicated through its FucR protein, which is unrelated to the *E. coli* FucR protein, to both induce the fucose operon and, along with FucR, to corepress an as-yet unidentified locus named *cps* (control of signal production) that may be responsible for inducing the mammalian host to make hydrolysable fucosylated glycans (24). Moreover, it has been shown that transcription of the ribose operon is repressed by XylR, the xylose regulator, in the presence of xylose (27). It is therefore not farfetched to suggest the possibility that in commensal *E. coli* strains fuculose-1-phosphate and FucR also regulate more than one operon. It also is possible that fuculose-1-phosphate interacts with the RbsR repressor protein or an as-yet unidentified protein to relieve repression of the ribose operon. Finally, it is possible that fuculose-1-phosphate is involved in opening a gate for ribose entry into the cells via a preexisting RbsABC transporter or in allosterically activating more of the ribose gene products.

It appears that accumulation of fuculose-1-phosphate inhibits its growth of both *E. coli* MG1655 Δ*fucAO* and *E. coli* Nissle 1917 Δ*fucAO* on mannose and galactose. This finding is not unexpected; i.e., it is well known that intracellular accumulation of sugar phosphates is inhibitory to *E. coli* growth, although the reasons remain unclear (6, 11, 13, 16, 17, 56). However, in view of the fact that sugar phosphates normally inhibit growth, it is unexpected that accumulation of fuculose-1-phosphate would stimulate growth of *E. coli* MG1655 Δ*fucAO* and *E. coli* Nissle 1917 Δ*fucAO* on ribose. Nevertheless, stimulation of ribose utilization by fuculose-1-phosphate appears to be a normal physiological process; i.e., fucose at a concentration too low to allow growth (0.005%) stimulated ribose utilization in both wild-type *E. coli* MG1655 and wild-type *E. coli* Nissle 1917 in vitro (Fig. 6 and 7C). In this context, although both fucose operons are activated by FucR in the presence of fuculose-1-phosphate (4, 9), it is possible that at low fucose concentrations the *fucPIKUR* operon is more highly expressed than the *fucAO* operon in wild-type *E. coli* MG1655, which could result in the accumulation of fuculose-1-phosphate. In fact, the two fucose operons can be expressed differentially (45, 57). The finding that low levels of fucose signal the wild-type strains to grow more rapidly on ribose raises the possibility that it also occurs in the intestine, perhaps in a minor niche. Such wild-type subpopulations presumably would be at the level of the population size of the Δ*fucAO* mutants in the presence of the wild-type strains, i.e., 100-fold below the

TABLE 1. Mouse intestinal colonization of *E. coli* Nissle 1917 mutants relative to that of wild-type *E. coli* Nissle 1917

Sugar	Defect in mutant	Input[a]	Difference between $\log_{10}$ CFU of the wild type and $\log_{10}$ CFU mutant on day: 3	9
Fucose[b]	Δ*fucAO*	0.13 ± 0.15	2.6 ± 0.5	2.3 ± 0.2
Ribose[b]	Δ*rbsK*	0.18 ± 0.15	0.03 ± 0.08	0.45 ± 0.23
Fucose, ribose[b]	Δ*fucAO* Δ*rbsK*	0.27 ± 0.33	2.6 ± 0.4	4.7 ± 0.4
Fucose[b]	Δ*fucK*	0.22 ± 0.18	0.60 ± 0.15	1.3 ± 0.1
Fucose[c]	Δ*fucAO*	4.7 ± 0.6	1.3 ± 0.3	1.7 ± 0.2
Fucose[c]	Δ*fucK*	4.5 ± 0.5	5.1 ± 0.4	5.5 ± 0.3

[a] Input values represent the mean of the $\log_{10}$ CFU of the *E. coli* Nissle 1917 mutant subtracted from the $\log_{10}$ CFU of the wild-type *E. coli* Nissle 1917 (± the $\log_{10}$ standard errors of the means) fed to six mice.

[b] Mice were fed 10^5 CFU each of an *E. coli* Nissle 1917 mutant and its wild-type parent. Mice were transferred to fresh cages every day, and feces no older than 24 h were assayed every other day for 15 days. At each time point, for each mouse the $\log_{10}$ CFU/gram of feces for the mutant was subtracted from the $\log_{10}$ CFU/gram of feces for the wild type. The $\log_{10}$ mean of the difference ± the $\log_{10}$ standard error of the mean of day 3 and day 9 fecal data from at least 6 mice are shown.

[c] The same procedure as that described in footnote *b* was followed, except that mice were fed 10^5 CFU of an *E. coli* Nissle 1917 mutant and 10^{10} CFU of its wild-type parent.

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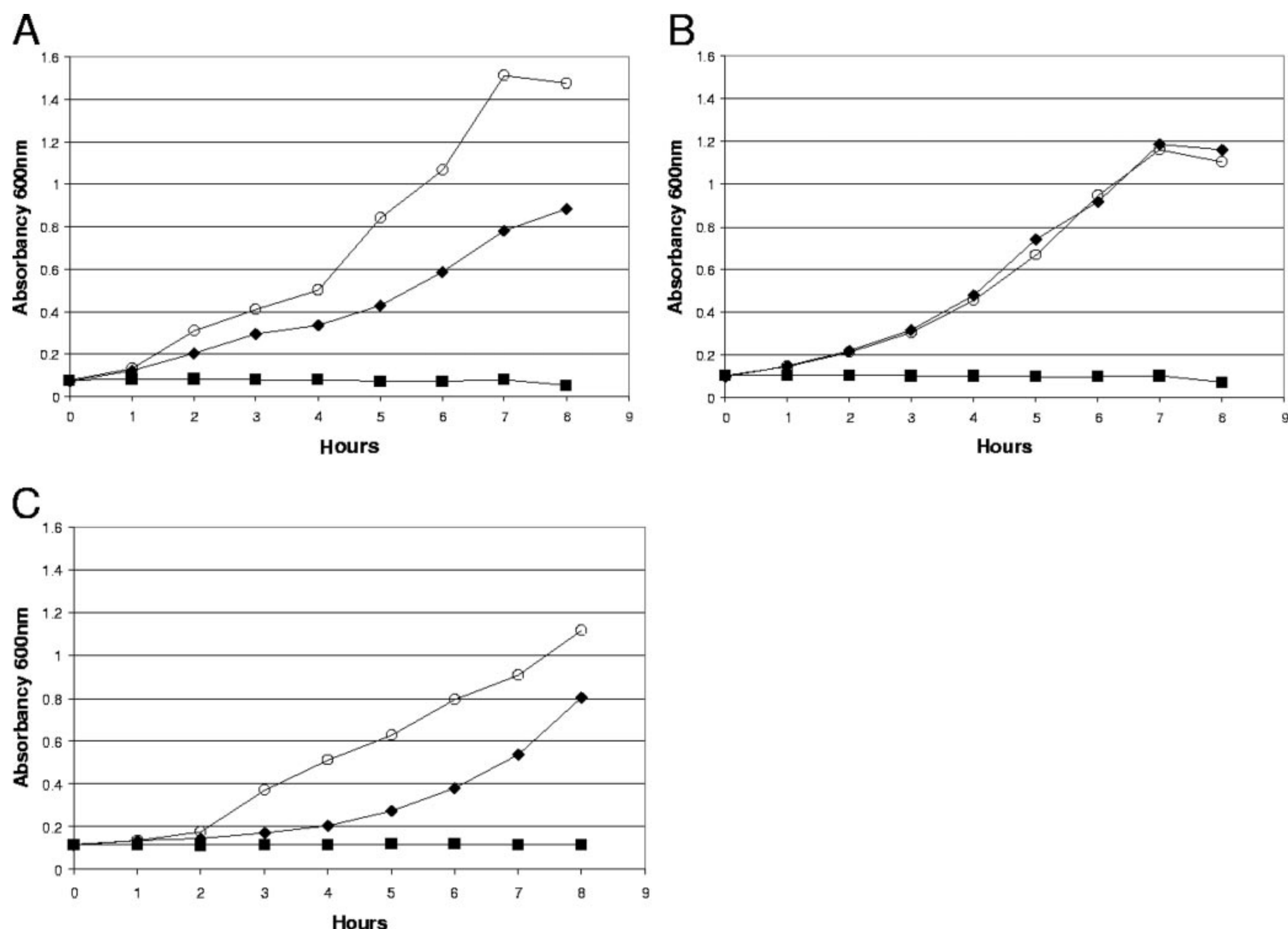

FIG. 7. Growth of *E. coli* Nissle 1917 Δ*fucAO* in M9 minimal medium in the presence of 0.05% (wt/wt) fucose (■), 0.15% (wt/wt) ribose (◆), or a mixture of 0.05% (wt/wt) fucose and 0.15% (wt/wt) ribose (○) (A); growth of *E. coli* Nissle 1917 Δ*fucK* in M9 minimal medium in the presence of 0.05% (wt/wt) fucose (■), 0.15% (wt/wt) ribose (◆), or a mixture of 0.05% (wt/wt) fucose and 0.15% (wt/wt) ribose (○) (B); and growth of wild-type *E. coli* Nissle 1917 in M9 minimal medium in the presence of 0.005% (wt/wt) fucose (■), 0.15% (wt/wt) ribose (◆), or a mixture of 0.005% (wt/wt) fucose and 0.15% (wt/wt) ribose (○) (C). Strains were grown in M9 minimal medium containing glycerol (0.2%, wt/wt), washed, and resuspended in M9 minimal medium containing the appropriate sugars (see Materials and Methods). Incubation was at 37°C with aeration. A_{600} readings at the indicated times are presented. Growth experiments were performed at least three times. The results of typical experiments are shown.

wild-type population (Fig. 2E), and would go unnoticed in routine colonization experiments. Experiments designed to test this possibility are currently in progress.

The finding that *E. coli* MG1655 Δ*fucAO* and *E. coli* Nissle 1917 Δ*fucAO* mutants switch to ribose in the intestine because they accumulate fuculose-1-phosphate not only supports the nutrient-niche theory but also has far-reaching implications with respect to the stability of the commensal flora in the intestine. *E. coli* and presumably other members of the intestinal microflora use several limiting nutrients simultaneously for growth (7, 10, 30, 31, 37), but how any member of the microflora chooses the specific nutrients to use at any one time among those available to it is largely unknown. The data presented here suggest that the intracellular pool size of a metabolic intermediate involved in the metabolism of one nutrient plays a role in this process by signaling *E. coli* to rapidly switch to a second unrelated nutrient for growth. Thus, it is possible that a variety of metabolic intermediates comprise a tier of regulation designed for *E. coli* and perhaps other members of the microflora to more efficiently utilize the available limiting multiple nutrients to maintain growth rates sufficient to avoid washout from the intestine.

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