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Escherichia coli F-18 and *E. coli* K-12 *eda* Mutants Do Not Colonize the Streptomycin-Treated Mouse Large Intestine

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The *Escherichia coli* human fecal isolates F-18 and K-12 are excellent colonizers of the streptomycin-treated mouse intestine. *E. coli* F-18 and *E. coli* K-12 *eda* mutants (unable to utilize glucuronate, galacturonate, and gluconate) were constructed by insertional mutagenesis. Neither the *E. coli* F-18 *eda* nor the *E. coli* K-12 *eda* mutant was able to colonize the streptomycin-treated mouse intestine, whether they were fed to mice together with their respective parental strains or alone. Complementation of the *eda* mutants with pTC190 (containing a functional *E. coli* K-12 *eda* gene) completely restored the colonization ability of both *eda* mutants. Relative to their parental strains, the *E. coli* F-18 *eda* mutant and the *E. coli* K-12 *eda* mutant grew poorly in cecal mucus isolated from mice fed either normal mouse chow or a synthetic diet containing sucrose as the sole carbon source, yet the mutants and parental strains demonstrated identical growth rates in minimal medium with glucose as the carbon source. *E. coli* F-18 *edd eda* and *E. coli* K-12 *edd eda* double mutants colonized the streptomycin-treated intestine when fed to mice alone; however, when fed simultaneously with their respective parental strains, they were poor colonizers. Since the *edd* gene is involved only in gluconate metabolism via the Entner-Doudoroff pathway, these results implicate the utilization of gluconate and the Entner-Doudoroff pathway as important elements in *E. coli* colonization of the streptomycin-treated mouse large intestine.

Escherichia coli F-18 was isolated from the feces of a healthy University of Rhode Island student in 1977 and is an excellent colonizer of the streptomycin-treated mouse large intestine (5, 24). *E. coli* K-12 was isolated from the feces of a convalescent diphtheria patient at Stanford University in 1922 (20) and is also an excellent colonizer of the streptomycin-treated mouse intestine (22). In the study described in the accompanying paper (27), we found that *E. coli* F-18 lacks *gntP*, one of four known gluconate permease genes in *E. coli* K-12, and that bacteriophage P1 transducing *gntP* from *E. coli* K-12 into *E. coli* F-18 gave it an advantage in the streptomycin-treated mouse large intestine. These results implicated gluconate in the ability of *E. coli* K-12 to colonize the streptomycin-treated mouse large intestine; however, since *E. coli* F-18 also metabolizes gluconate and since it is possible that the gluconate permease encoded by *gntP* transports another sugar in the mouse intestine, we decided to investigate the role of gluconate in *E. coli* colonization of the streptomycin-treated mouse large intestine in a more systematic way.

MATERIALS AND METHODS

Bacteria and plasmids. A summary of the *E. coli* strains and plasmids used in the present investigation is presented in Table 1.

DNA techniques. *E. coli* HB101 (4) was transformed with plasmids via electroporation (7) with a gene pulser (Bio-Rad Laboratories, Richmond, Calif.). Plasmids were isolated from transformed *E. coli* HB101 with Promega Magic Miniprep columns (Promega Corp., Madison, Wis.) and were subsequently electroporated into *E. coli* F-18 and *E. coli* K-12 strains.

Construction of *eda*::miniTn*10*::*npt* and *edd eda*::miniTn*10*::*npt* *E. coli* mutants. An *E. coli* F-18 *eda*::miniTn*10*::*npt* mutant was constructed by insertional mutagenesis (6, 18). Briefly, *E. coli* ATM160 carrying the suicide vector pLOF, which contains the miniTn*10*::*npt* transposon, was conjugated with the recipient *E. coli* F-18 strain in the following manner. Donor and recipient strains were grown overnight in Luria broth with shaking at 30°C. Aliquots of 100 μl of each culture were mixed in 5 ml of 10 mM $MgSO_4$ and filtered through a Millipore 0.45-μm-pore-size membrane filter. The filter was placed on the surface of a Luria agar plate and incubated for 5 h at 37°C. Following incubation, the bacteria on the filter were suspended in 5 ml of 10 mM $MgSO_4$, and 100-μl aliquots of the suspension were plated on MacConkey agar (Difco Laboratories, Detroit, Mich.) containing streptomycin sulfate (100 μg/ml) and kanamycin sulfate (80 μg/ml), with glucuronate (2.5 mg/ml) as the carbohydrate source. Glucuronate fermenters appear red on MacConkey agar, and nonfermenters appear white. White colonies obtained from the MacConkey-glucuronate plates were then screened for their ability to utilize the carbohydrates glucose, glucuronate, galacturonate, gluconate, and galactonate by being streaked onto Davis minimal agar (Difco) plates containing either glucose (1.0 mg/ml), glucuronate (2.5 mg/ml), galacturonate (2.5 mg/ml), gluconate (1.0 mg/ml), or galactonate (1.0 mg/ml) as the sole carbon source; the plates were incubated at 30°C for 48 h and observing for growth. *E. coli uxu* mutants cannot utilize glucuronate but can utilize all of the other carbohydrates listed above (Fig. 1). *E. coli eda* mutants cannot utilize glucuronate, galacturonate, or gluconate but can utilize glucose and galactonate (Fig. 1). Presumptive *E. coli* F-18 *eda*::miniTn*10*::*npt* mutants were screened for sensitivity to ampicillin (to eliminate the possibility of pLOF integration into the chromosome) by observing the lack of growth on MacConkey agar containing lactose (MacConkey-lactose agar), streptomycin sulfate (100 μg/ml), and ampicillin (100 μg/ml). All *E. coli* strains used in this study grow well on MacConkey-lactose agar in the absence of antibiotics.

E. coli edd eda::miniTn*10*::*npt* double mutants were selected by plating 100-μl aliquots of a Luria broth-grown (37°C for 18 h) culture of the *E. coli eda*::miniTn*10*::*npt* strain onto bromthymol blue (BTB)-gluconate agar plates (19), incubating overnight at 37°C, and selecting the green colonies (3).

Bacteriophage P1 transduction. All P1 transductions and curing transductants of P1 were performed as previously described (22). *E. coli* K-12 *eda*::miniTn*10*::*npt* was constructed by infecting *E. coli* K-12 Str^r with the P1 lysate from an induced Luria broth culture of lysogenized *E. coli* F-18 *eda*::miniTn*10*::*npt* and selecting colonies on Luria agar containing streptomycin sulfate (100 μg/ml) and kanamycin sulfate (100 μg/ml). The *eda*::miniTn*10*::*npt* mutation in the resulting transductant was confirmed by its inability to utilize glucuronate, galacturonate, and gluconate, as described above.

In vitro growth in mouse cecal mucus. Mouse cecal mucus was isolated as previously described (28). Briefly, to investigate the role of diet on growth in cecal mucus, 18 mice were fed Charles River Valley rat, mouse, and hamster formula for 5 days and 18 were fed purified mouse diet (ICN Biomedicals, Costa Mesa, Calif.), which contains sucrose as the sole carbon source. The drinking water was then replaced with sterile distilled water containing streptomycin sulfate (5 g/liter). Twenty four hours later, the mice were sacrificed by CO_2 asphyxiation and their ceca were removed. The cecal contents were washed out of each cecum with sterile *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-Hanks buffer (pH 7.4), and the cecal mucus was scraped from the washed cecum. The cecal mucus obtained from the mice was pooled and mixed to obtain a homogeneous, highly viscous sample.

The ceca from each set of 18 mice yielded approximately 2 ml of viscous

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TABLE 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Relevant characteristics[a]	Source or reference
Strains		
F-18	Human commensal strain, Str^r Rif^r	24
F-18 Nal^r	Nalidixic acid-resistant mutant of F-18	22
F-18 *eda*::miniTn*10*::*npt*	miniTn*10*::*npt* insertion into *eda*	This study
F-18 *edd eda*::miniTn*10*::*npt*	*edd* mutant of F-18 *eda*::miniTn*10*::*npt*	This study
K-12	Wild type	20
K-12 Str^r	Streptomycin-resistant mutant of K-12	This study
K-12 Str^r *eda*::miniTn*10*::*npt*	Transduction of miniTn*10*::*npt* insertion into *eda* from F-18 into K-12 Str^r	This study
K-12 Str^r *edd eda*::miniTn*10*::*npt*	*edd* mutant of K-12 Str^r *eda*::miniTn*10*	This study
Plasmids		
pBluescriptII	Amp^r	Stratagene
pTC180	*edd* cloned into pBluescriptII	8
pTC190	*eda* cloned into pBluescriptII	8

[a] *npt*, gene conferring resistance to kanamycin.

mucus. Two 1-ml aliquots were then separately inoculated with 10^4 CFU of either *E. coli* F-18 Nal^r or *E. coli* F-18 *eda*::miniTn*10*::*npt* in one set of experiments and inoculated with 10^4 CFU of *E. coli* K-12 Str^r or *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt* in a separate set of experiments. All input *E. coli* strains were grown in Luria broth (at 37°C with shaking, for 18 h), centrifuged, washed three times in sterile HEPES-Hanks buffer, and diluted in HEPES-Hanks buffer. Cecal mucus samples were incubated standing at 37°C. Samples were withdrawn at 0, 2, 4, and 6 h, diluted in 1% tryptone broth (Difco), and plated on MacConkey-lactose agar containing selective antibiotics. *E. coli* F-18 Nal^r was enumerated on MacConkey-lactose agar containing 100 μg of streptomycin sulfate per ml and 50 μg of rifampin per ml. *E. coli* F-18 *eda*::miniTn*10*::*npt* was enumerated on MacConkey-lactose agar containing 100 μg of streptomycin sulfate per ml and 80 μg of kanamycin sulfate per ml. *E. coli* K-12 Str^r was plated on MacConkey-lactose agar containing streptomycin sulfate (100 μg/ml), and *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt* was plated on MacConkey-lactose agar containing streptomycin sulfate (100 μg/ml) and kanamycin sulfate (80 μg/ml). All MacConkey-lactose plates were incubated at 37°C for 18 to 24 hours before being counted.

Mouse colonization experiments. The method used to compare the relative large intestine-colonizing abilities of *E. coli* strains in mice has been described previously (5, 28). Briefly, three male CD-1 mice (5 to 8 weeks old) were given streptomycin sulfate-treated (5 g/liter) drinking water for 24 h. Following 18 to 24 h of starvation, the mice were fed 1 ml of 20% (wt/vol) sucrose containing 10^{10} CFU of Luria broth-grown *E. coli*. The strains were fed to mice either alone or along with another strain. After the bacterial suspension had been ingested, both the food (Charles River Valley rat, mouse, and hamster formula) and streptomycin-water were returned to the mice; 1 g of feces was collected after 5 and 24 h and on odd-numbered days for 2 weeks thereafter. Fecal samples (no older than 24 h) were homogenized, diluted in 1% tryptone broth, and plated on MacConkey-lactose agar containing antibiotics as indicated in the legends to the figures to enumerate each *E. coli* strain. Antibiotic concentrations on plates were as follows: streptomycin sulfate, 100 μg/ml; rifampin, 50 μg/ml; tetracycline hydrochloride, 10 μg/ml; kanamycin sulfate, 80 μg/ml; nalidixic acid, 50 μg/ml; ampicillin, 100 μg/ml. All the plates were incubated for 18 to 24 h at 37°C before being counted. Each colonization experiment was repeated twice to confirm initial colonization results. All plates were incubated for 18 to 24 h at 37°C. The $\log_{10}$ mean number of CFU per gram of feces ± the standard error (SE) for each strain in the mice was calculated for each time point.

If the concentration of an *E. coli* strain in mouse feces was below the detection

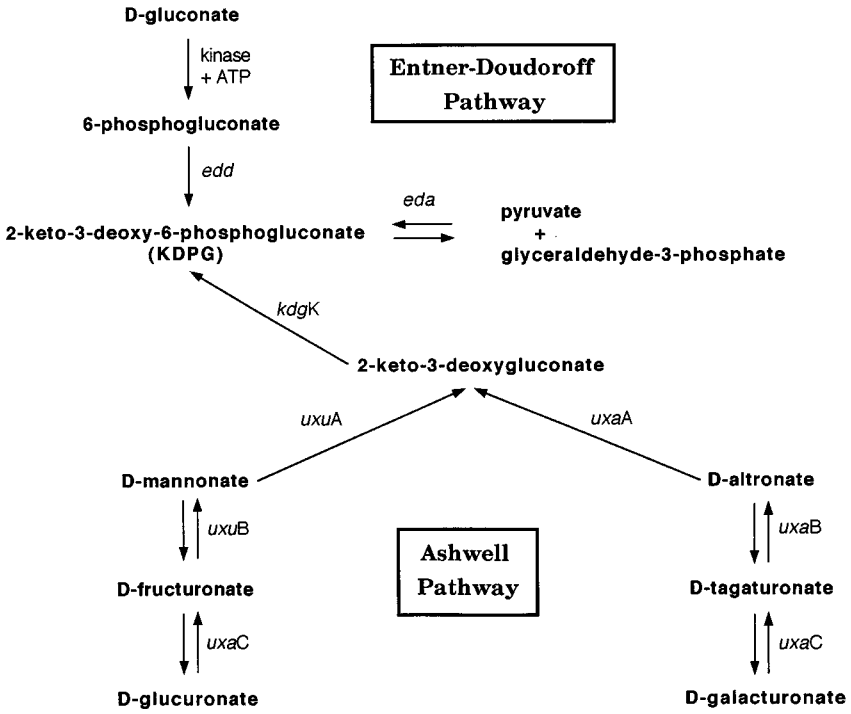

FIG. 1. Degradative pathways of D-gluconate, D-glucuronate, and D-galacturonate by *E. coli.*

TABLE 2. Growth of *E. coli* strains with various sugars as sole carbon sources

E. coli strain	Growth on[a]:				
	Glc	GlcA	GalA	Gnt	Galnt
F-18	+	+	+	+	+
F-18 Nal^r	+	+	+	+	+
F-18 *eda*::miniTn*10*::*npt*	+	0	0	0	+
F-18 *eda*::miniTn*10*::*npt*(pTC190)	+	+	+	+	+
F-18 *eda*::miniTn*10*::*npt*(pBluescriptII)	+	0	0	0	+
F-18 *edd eda*::miniTn*10*::*npt*	+	0	0	0	+
F-18 *edd eda*::miniTn*10*::*npt*(pTC180)	+	0	0	0	+
F-18 *edd eda*::miniTn*10*::*npt*(pTC190)	+	+	+	0	+
F-18 *edd eda*::miniTn*10*::*npt*(pBluescriptII)	+	0	0	0	+
K-12 Str^r	+	+	+	+	+
K-12 Str^r *eda*::miniTn*10*::*npt*	+	0	0	0	+
K12 Str^r *eda*::miniTn*10*::*npt*(pTC190)	+	+	+	+	+
K-12 Str^r *eda*::miniTn*10*::*npt*(pBluescriptII)	+	0	0	0	+
K-12 Str^r *edd eda*::miniTn*10*::*npt*	+	0	0	0	+
K-12 Str^r *edd eda*::miniTn*10*::*npt*(pTC180)	+	0	0	0	+
K-12 Str^r *edd eda*::miniTn*10*::*npt*(pTC190)	+	+	+	0	+
K-12 Str^r *edd eda*::miniTn*10*::*npt*(pBluescriptII)	+	0	0	0	+

[a] Glc, glucose (1.0 mg/ml); GlcA, glucuronate (2.5 mg/ml); GalA, galacturonate (2.5 mg/ml); Gnt, gluconate (1.0 mg/ml); Galnt, galactonate (1.0 mg/ml). Davis minimal agar plates were incubated at 30°C for 48 h.

limit by the end of the experiment, the mice were sacrificed and their cecal mucus was isolated as described above. The mucus from the cecum of each mouse (approximately 100 μl) was plated onto MacConkey-lactose agar containing the appropriate antibiotics to determine if the *E. coli* strain could be detected in mouse cecal mucus.

RESULTS

***E. coli eda*::miniTn*10*::*npt* and *edd eda*::miniTn*10*::*npt* mutants.** *E. coli* utilizes the gene products of the *uxu* genes (26) to metabolize glucuronate via the Ashwell pathway (2) and the *edd* and *eda* gene products (9, 13) to metabolize gluconate via the Entner-Doudoroff pathway (10). Gluconate, glucuronate, and galacturonate are converted to 2-keto-3-deoxy-6-phosphogluconate (KDPG), the point at which the Entner-Doudoroff and Ashwell pathways converge (Fig. 1). KDPG is cleaved by KDPG aldolase (encoded by *eda*) to yield pyruvate and glyceraldehyde 3-phosphate. Thus, isolation of mutants unable to utilize glucuronate on MacConkey agar (white colonies [wild-type colonies are red]) results in the selection of *eda* mutants as well. This was necessary, since *E. coli eda* mutants grow on MacConkey agar containing glucuronate but not gluconate.

A summary of utilization of selected carbohydrates as sole carbon sources by *E. coli eda*::miniTn*10*::*npt* and *edd eda*::miniTn*10*::*npt* mutants and their corresponding parental strains is presented in Table 2. *E. coli* F-18 *eda*::miniTn*10*::*npt* and *E. coli* K-12 *eda*::miniTn*10*::*npt* did not grow on gluconate, glucuronate, or galacturonate but grew on glucose and galactonate as sole carbon sources. Utilization of gluconate, glucuronate, and galacturonate via the Ashwell and Entner-Doudoroff pathways requires a functional *eda* gene product (Fig. 1). When the *E. coli eda*::miniTn*10*::*npt* mutants were complemented with pTC190 (containing a functional *eda* gene), utilization of these carbohydrates was restored (Table 2). In contrast, the control vector pBluescriptII did not restore gluconate, glucuronate, and galacturonate utilization as sole carbon sources, thereby confirming the *eda*::miniTn*10*::*npt* mutations (Table 2).

BTB-gluconate plates were used to differentiate *E. coli eda*::miniTn*10*::*npt* mutants from *edd eda*::miniTn*10*::*npt* double mutants. Despite containing peptones and yeast extract, BTB-gluconate plates do not support the growth of *E. coli eda*::miniTn*10*::*npt* mutants, presumably because of an accumulation of KDPG, which is known to be toxic to *E. coli* (11, 16). *E. coli edd eda*::miniTn*10*::*npt* mutants grow on BTB-gluconate plates because the *edd* mutation prevents the formation of KDPG. The *edd eda*::miniTn*10*::*npt* double mutants were confirmed by the observation that when these mutants were transformed with plasmid pTC180, containing the functional *E. coli* K-12 *edd* gene, the transformants did not grow on the BTB-gluconate plates, again presumably because of the accumulation of KDPG. *E. coli edd eda*::miniTn*10*::*npt* mutants transformed with the control vector pBluescriptII grew on BTB-gluconate plates.

Glucuronate, galacturonate, and gluconate degradation should each result in the formation of the growth-inhibitory intermediate KDPG (Fig. 1). As stated above, the *E. coli eda*::miniTn*10*::*npt* mutants did not grow on MacConkey agar containing gluconate but did grow on MacConkey agar containing glucuronate. Moreover, the *E. coli eda*::miniTn*10*::*npt* mutants grew on MacConkey agar plates containing galacturonate. Therefore, gluconate inhibited the *E. coli eda*::miniTn*10*::*npt* mutants from utilizing the other carbon sources present in MacConkey agar, but neither glucuronate nor galacturonate prevented these mutants from growing on MacConkey agar; i.e., the *eda*::miniTn*10*::*npt* mutants grew and appeared white, suggesting that metabolism of glucuronate and galacturonate does not, for presently unknown reasons, result in accumulation of growth-inhibitory amounts of KDPG in *eda* mutants.

Relative colonizing abilities of *E. coli* F-18 Nal^r and *E. coli* F-18 *eda*::miniTn*10*::*npt* and of *E. coli* K-12 Str^r and *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt*. When mice were fed 10^{10} CFU each of *E. coli* F-18 Nal^r and *E. coli* F-18 *eda*::miniTn*10*::*npt*, *E. coli* F-18 colonized at approximately 10^8 CFU/g of feces whereas *E. coli* F-18 *eda*::miniTn*10*::*npt* was essentially eliminated from the intestine by day 3 (Fig. 2A). On day 15 postfeeding, the mice were sacrificed and 100 μl of cecal mucus from each animal was plated on MacConkey-lactose agar plates containing streptomycin, rifampin, and kanamycin. *E. coli* F-18 *eda*::miniTn*10*::*npt* colonies were not detected (<1 CFU in the mucus of the entire cecum). Of greater interest, when 10^{10} CFU of *E. coli* F-18 *eda*::miniTn*10*::*npt* was fed to streptomycin-treated mice alone, it failed to colonize the

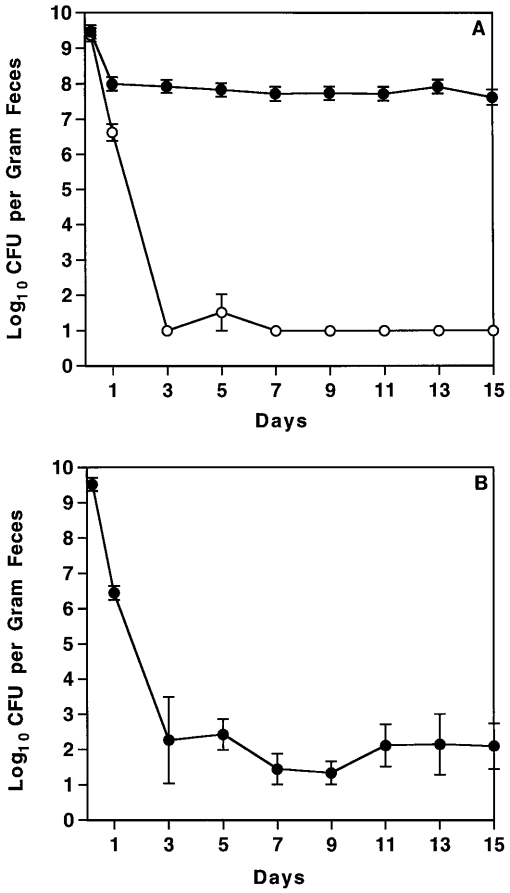

FIG. 2. Colonization of *E. coli* F-18 *eda*::miniTn*10*::*npt* and *E. coli* F-18 Nal^r in the streptomycin-treated mouse. Three streptomycin-treated mice were fed 10^{10} CFU of *E. coli* F-18 Nal^r (●) and 10^{10} CFU of *E. coli* F-18 *eda*::miniTn*10*::*npt* (○) (A) or 10^{10} CFU of *E. coli* F-18 *eda*::miniTn*10*::*npt* (B). Platings were done on MacConkey-lactose agar plates containing either streptomycin, rifampin, and nalidixic acid to enumerate *E. coli* F-18 Nal^r or streptomycin, rifampin, and kanamycin to enumerate *E. coli* F-18 *eda*::miniTn*10*::*npt.*

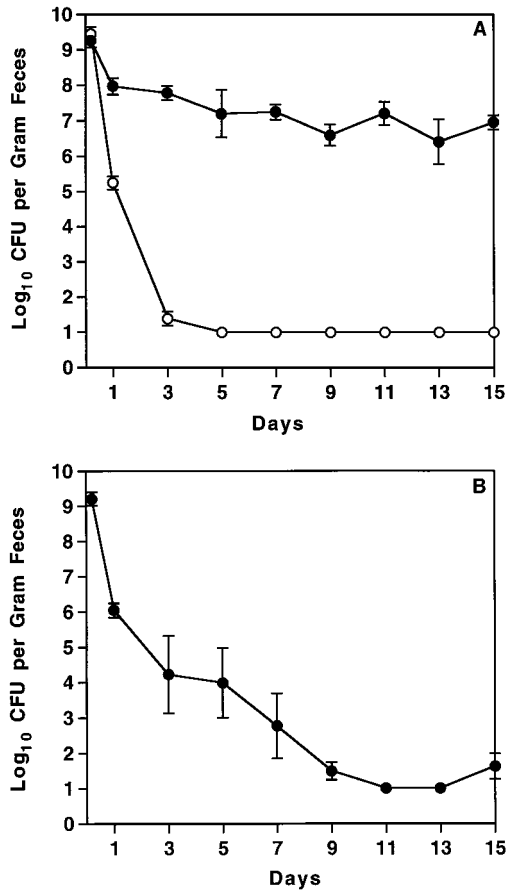

FIG. 3. Colonization of *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt* and *E. coli* K-12 Str^r in the streptomycin-treated mouse. Three streptomycin-treated mice were fed 10^{10} CFU of *E. coli* K-12 Str^r (●) and 10^{10} CFU of *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt* (○) (A) or 10^{10} CFU of *E. coli* F-18 *eda*::miniTn*10*::*npt* (B). Platings were done on MacConkey-lactose agar plates containing either streptomycin to enumerate *E. coli* K-12 Str^r or streptomycin and kanamycin to enumerate *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt.*

mouse intestine (Fig. 2B). Similarly, *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt* was unable to colonize the mouse intestine either alone or in the presence of its parent (Fig. 3). Furthermore, *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt* was not found in cecal mucus after disappearing from the feces (<1 CFU in the mucus of the entire cecum).

The only non-pBluescriptII gene that pTC190 contains is a functional *E. coli* K-12 *eda* gene (8). Complete colonization ability was restored when *E. coli* F-18 *eda*::miniTn*10*::*npt* was complemented with pTC190. Indeed, when *E. coli* F-18 *eda*::miniTn*10*::*npt*(pTC190) was fed (10^{10} CFU) to streptomycin-treated mice, it colonized at approximately 5×10^7 CFU/g of feces (Fig. 4A). In a control experiment, a separate set of mice were fed 10^{10} CFU of *E. coli* F-18 *eda*::miniTn*10*::*npt*(pBluescriptII). This strain was essentially eliminated by day 3 postfeeding (Fig. 4B). The fecal samples in each of these experiments were plated on MacConkey-lactose agar containing streptomycin sulfate (100 μg/ml) and kanamycin (80 μg/ml) to enumerate total CFU and on MacConkey-lactose agar containing streptomycin sulfate (100 μg/ml) and ampicillin (100 μg/ml) to enumerate plasmid-containing CFU. In each case, the number of total CFU was nearly identical to the number of plasmid-containing CFU (data not shown). Similarly, *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt*(pTC190) regained almost full colonizing ability but *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt*(pBluescriptII) did not (Fig. 5). Collectively, these data suggest that a functional *eda* is required for both *E. coli* F-18 and *E. coli* K-12 Str^r to colonize the streptomycin-treated mouse large intestine.

Relative colonizing abilities of *E. coli* F-18 Nal^r and *E. coli* F-18 *edd eda*::miniTn*10*::*npt* and of *E. coli* K-12 Str^r and *E. coli* K-12 Str^r *edd eda*::miniTn*10*::*npt.* As demonstrated in Table 2, an *edd* mutation in *E. coli* F-18 *eda*::miniTn*10*::*npt* relieved the inhibition of growth on gluconate, presumably because it prevents the accumulation of the growth-inhibiting compound KDPG (8). To determine whether the *edd* mutation might also improve the colonizing ability of *E. coli* F-18 *eda*::miniTn*10*::*npt*, mice were fed 10^{10} CFU of *E. coli* F-18 *edd eda*::miniTn*10*::*npt*. This strain colonized at approximately 10^8 CFU/g of feces (Fig. 6A). However, when mice were fed 10^{10} CFU of *E. coli* F-18 *edd eda*::miniTn*10*::*npt* and 10^{10} CFU of *E. coli* F-18 Nal^r, *E. coli* F-18 Nal^r colonized at approximately 10^9 CFU/g of feces whereas *E. coli* F-18 *edd eda*::miniTn*10*::*npt* colonized at a level of only 10^4 CFU/g of feces (Fig. 6B). Similarly, *E. coli* K-12 Str^r *edd eda*::miniTn*10*::*npt* colonized better than *E. coli* K-12 *eda*::miniTn*10*::*npt* when fed alone to mice (Fig. 7A) but was unable to colonize the mouse intestine to any great extent in

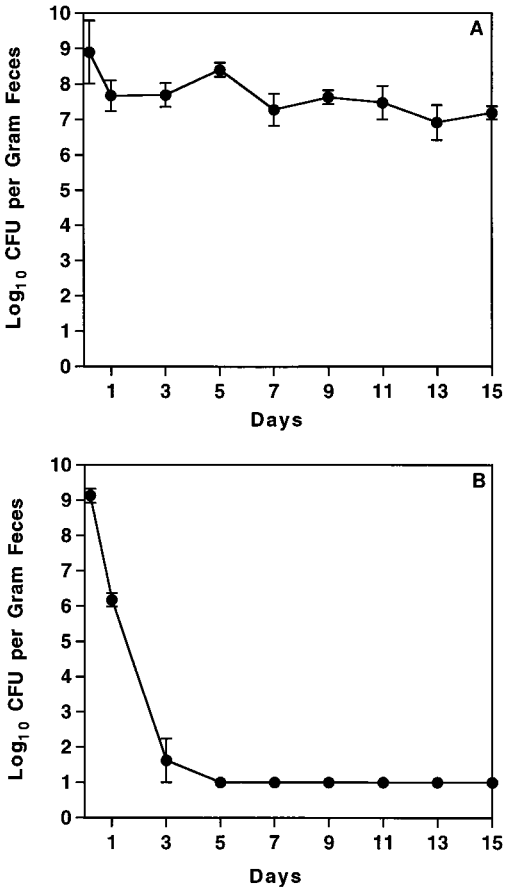

FIG. 4. Colonization of *E. coli* F-18 *eda*::miniTn*10*::*npt*(pTC190) and *E. coli* F-18 *eda*::miniTn*10*::*npt*(pBluescriptII) in the streptomycin-treated mouse. Three streptomycin-treated mice were fed 10^{10} CFU of *E. coli* F-18 *eda*::miniTn*10*::*npt*(pTC190) (A) or 10^{10} CFU of *E. coli* F-18 *eda*::miniTn*10*::*npt* (pBluescriptII) (B). Platings were done on MacConkey-lactose agar plates containing streptomycin and ampicillin.

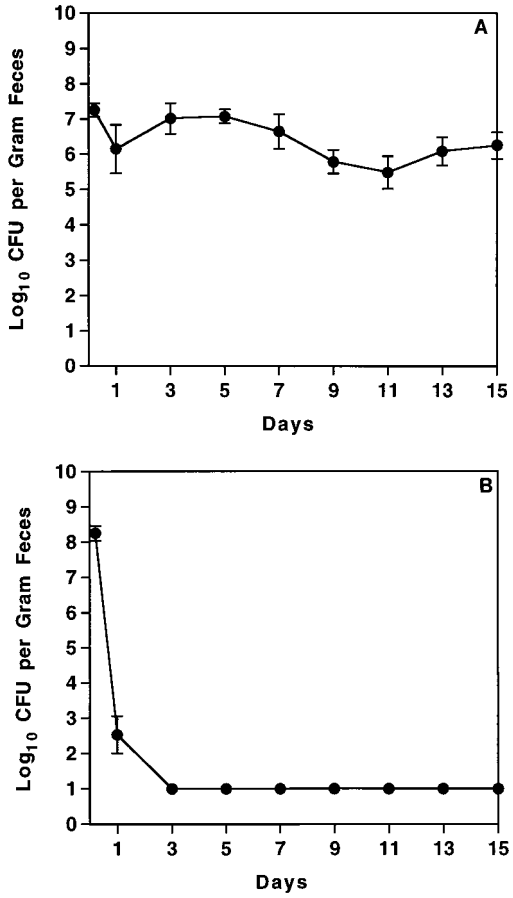

FIG. 5. Colonization of *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt*(pTC190) and *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt*(pBluescriptII) in the streptomycin-treated mouse. Three streptomycin-treated mice were fed 10^{10} CFU of *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt*(pTC190) (A) or 10^{10} CFU of *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt*(pBluescriptII) (B). Platings were done on MacConkey-lactose agar plates containing streptomycin and ampicillin.

the presence of its parent (Fig. 7B). Since gluconate is the only known carbon source that utilizes the *edd* gene product, these data suggest that the functioning of the Entner-Doudoroff pathway is required for maximum colonizing ability.

In vitro growth in mouse cecal mucus. *E. coli* F-18 grows in cecal mucus in vitro but does not grow in cecal contents in vitro, suggesting that the ability to colonize depends on the ability to grow in the mucus layer of the streptomycin-treated mouse large intestine (28). Crude cecal mucus specimens isolated from mice fed either a normal laboratory chow diet or a synthetic diet containing only sucrose as the carbon source were separately inoculated with either *E. coli* F-18 *eda*::miniTn*10*::*npt* or *E. coli* F-18 Nal^r as described in Materials and Methods. After 6 h of incubation in cecal mucus isolated from mice on the normal laboratory chow diet, *E. coli* F-18 *eda*::miniTn*10*::*npt* had not grown whereas *E. coli* F-18 Nal^r had grown about 100-fold (Table 3). Similar results were obtained when cecal mucus isolated from mice fed the synthetic diet containing sucrose as the sole carbon source; i.e., when cecal mucus isolated from these mice was separately inoculated with either *E. coli* F-18 Nal^r or *E. coli* F-18 *eda*::miniTn*10*::*npt*, *E. coli* F-18 Nal^r grew about 2,000-fold in 6 h whereas *E. coli* F-18 *eda*::miniTn*10*::*npt* barely doubled in number in the same time (Table 4). Nearly identical results were obtained with *E. coli* K-12 Str^r and *E. coli* K-12 Str^r *eda*::miniTn*10*::*npt* (data not shown). All four strains grew equally well and with equal generation times in Davis minimal medium containing glucose as the carbon source (data not shown). These results therefore suggest that enough gluconate is present in cecal mucus even in the absence of gluconate in the diet to act as a carbon source for both *E. coli* F-18 and *E. coli* K-12 Str^r growth in the intestine.

DISCUSSION

The data presented here demonstrate the requirement of a functional *eda* gene for *E. coli* F-18 and *E. coli* K-12 to colonize the streptomycin-treated mouse intestine. That is, neither *E. coli* F-18 nor *E. coli* K-12 *eda* mutants colonized the mouse intestine to any great extent when fed alone to mice (Fig. 2B and 3B). However, when the *eda* mutants were complemented with a wild-type *eda* gene, their colonizing ability was restored (Fig. 4A and 5A).

The product of the *eda* gene, KDPG aldolase, is used in the metabolism of glucuronate, galacturonate, and gluconate (Fig. 1). Although we have no information as yet on the importance of glucuronate and galacturonate, we have shown that gluconate appears to be a carbon source for *E. coli* colonization of

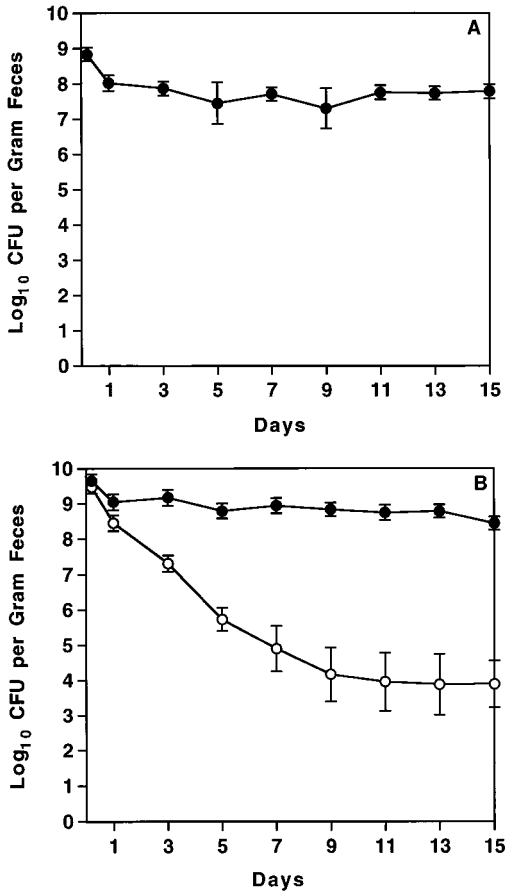

FIG. 6. Colonization of *E. coli* F-18 *edd eda*::miniTn*10*::*npt* and *E. coli* F-18 Nal^r in the streptomycin-treated mouse. Three streptomycin-treated mice were fed 10^{10} CFU of *E. coli* F-18 *edd eda*::miniTn*10*::*npt* (A) or 10^{10} CFU of *E. coli* F-18 Nal^r (●) and 10^{10} CFU of *E. coli* F-18 *edd eda*::miniTn*10*::*npt* (○) (B). Platings were done on MacConkey-lactose agar plates containing either streptomycin, rifampin, and nalidixic acid to enumerate *E. coli* F-18 Nal^r or streptomycin, rifampin, and kanamycin to enumerate *E. coli* F-18 *edd eda*::miniTn*10*::*npt.*

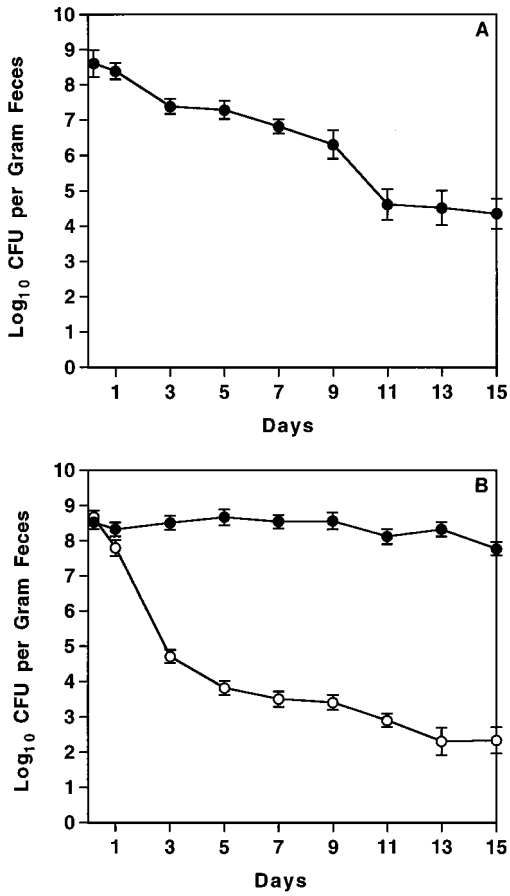

FIG. 7. Colonization of *E. coli* K-12 Str^r *edd eda*::miniTn*10*::*npt* and *E. coli* K-12 Str^r in the streptomycin-treated mouse. Three streptomycin-treated mice were fed 10^{10} CFU of *E. coli* K-12 Str^r *edd eda*::miniTn*10*::*npt* (A) or 10^{10} CFU of *E. coli* K-12 Str^r (●) and 10^{10} CFU of *E. coli* K-12 Str^r *edd eda*::miniTn*10*::*npt* (○) (B). Platings were done on MacConkey-lactose agar plates containing either streptomycin to enumerate *E. coli* K-12 Str^r or streptomycin and kanamycin to enumerate *E. coli* K-12 Str^r *edd eda*::miniTn*10*::*npt.*

the streptomycin-treated mouse large intestine. That is, although *E. coli* F-18 and *E. coli* K-12 *eda* mutants failed to colonize when fed to mice alone, presumably because of KDPG accumulation (11, 16), the *edd eda* double mutants colonized well (Fig. 6A and 7A). Since the *edd* mutation relieves only KDPG accumulation caused by metabolism of gluconate in an *eda* mutant (Fig. 1), gluconate is implicated as a carbon source for *E. coli* in the intestine. In support of this view, while both the *E. coli* F-18 and *E. coli* K-12 *edd eda* double mutants were able to colonize alone, they were poor colonizers when fed to mice together with their wild-type parents (Fig. 6B and 7B). These data suggest that when an *E. coli* strain is unable to utilize the Entner-Doudoroff pathway in the intestine, even if inhibition by gluconate-derived KDPG is blocked by an *edd* mutation, the strain is unable to grow as rapidly in the intestine as one that has that ability.

At present, we do not know which carbon source or which pathway the *edd eda* mutants utilize for growth in and colonization of the streptomycin-treated mouse large intestine. However, it is clear that glucuronate and galacturonate are not being utilized, since their metabolism requires a functional *eda* gene to produce pyruvate and glyceraldehyde-3-phosphate for energy (2) (Fig. 1). It is possible, however, that gluconate is also being utilized in *edd eda* double mutants, since gluconate metabolism is not confined to the Entner-Doudoroff pathway but can proceed via the hexose monophosphate shunt, although growth is slower under such conditions (29).

E. coli F-18 grows in cecal mucus in vitro but does not grow in cecal luminal contents (28), suggesting that the ability to colonize depends on the ability to grow in the mucus layer of the intestine. In support of this view, both the *E. coli* F-18 *eda* and *E. coli* K-12 *eda* mutants grew poorly in mucus in vitro

TABLE 3. Growth of *E. coli* F-18 Nal^r and *E. coli* F-18 *eda*::miniTn*10*::*npt* in cecal mucus isolated from mice on a conventional diet

Time (h)	*E. coli* growth (CFU/ml)		Ratio[a]
	F-18 Nal^r	F-18 *eda*::miniTn*10*::*npt*	
0	2.3×10^4	2.4×10^4	0.96
2	1.0×10^5	3.4×10^4	2.94
4	1.3×10^6	3.6×10^4	36.11
6	3.2×10^6	3.2×10^4	100.00

[a] Ratio of growth of *E. coli* F-18 Nal^r to growth of *E. coli* F-18 *eda*::miniTn*10*::*npt.*

TABLE 4. Growth of *E. coli* F-18 Nal^r and *E. coli* F-18 *eda*::miniTn*10*::*npt* in cecal mucus isolated from mice on a purified sucrose diet

Time (h)	*E. coli* growth (CFU/ml)		Ratio[a]
	F-18 Nal^r	F-18 *eda*::miniTn*10*::*npt*	
0	5.2×10^3	1.2×10^4	0.43
2	4.1×10^4	2.3×10^4	1.78
4	1.0×10^6	2.8×10^4	35.71
6	1.2×10^7	2.9×10^4	413.00

[a] Ratio of growth of *E. coli* F-18 Nal^r to growth of *E. coli* F-18 *eda*::miniTn*10*::*npt*.

(Table 3) and failed to colonize the mouse intestine (Fig. 2B and 3B). In addition, the *E. coli* F-18 *eda* mutant grew poorly in cecal mucus isolated from mice on a synthetic diet containing sucrose as the sole carbon source (Table 4). Therefore, it appears that endogenous rather than dietary gluconate in cecal mucus is the carbon source for *E. coli* F-18 and *E. coli* K-12 growth in and colonization of the streptomycin-treated mouse large intestine.

As stated above, in the presence of gluconate, the Entner-Doudoroff intermediate KDPG accumulates in *E. coli eda* mutants (Fig. 1), and it is well known that an intracellular accumulation of KDPG has growth-inhibitory properties (11, 16). The inhibitory nature of gluconate on the *eda* mutants was demonstrated by the inability of *E. coli* F-18 *eda*::miniTn*10*::*npt* and *E. coli* K-12 *eda*::miniTn*10*::*npt* to grow on BTB-gluconate agar and on MacConkey agar containing gluconate. Each of these growth media contains carbon sources other than gluconate, but the presence of gluconate prevents growth. In contrast, *E. coli eda* mutants exposed to MacConkey agar containing either glucuronate or galacturonate as the carbon source do grow, although they cannot ferment these carbohydrates and should accumulate KDPG. KDPG is produced by Ashwell pathway enzymes in the degradation of hexuronates (2) and by Entner-Doudoroff pathway enzymes in the degradation of gluconate (12). Therefore, it is possible that the Ashwell pathway is regulated such that growth-inhibitory concentrations of KDPG do not accumulate in the presence of glucuronate and galacturonate.

The conventional mammalian large intestine is populated by 400 to 500 species of bacteria, each maintaining a numerical balance in the presence of the others (23). The prevailing theory of how this balance is maintained is that each microorganism is able to utilize a growth-limiting nutrient better than the others (14, 15, 21). An exception to this theory is that two microorganisms can use the same nutrient and survive in the intestine as long as the one that uses it less efficiently can adhere to epithelial cells and thereby prevent washout (14, 15). At present, we do not know whether *E. coli* F-18 and *E. coli* K-12 utilize gluconate better than any of the myriad of species in the streptomycin-treated mouse large intestine or whether they use it less efficiently than another species but adhere to epithelial cells and thereby remain in the intestine. However, we favor the former hypothesis, since recent evidence shows that *E. coli* BJ4, also a commensal strain, is dispersed throughout the cecal mucus layer but does not appear to adhere to epithelial cells in streptomycin-treated mice (25).

A major problem encountered in studying the colonization of the mammalian intestine is what is commonly referred to as "colonization resistance," in which all intestinal niches are occupied in a balanced ecosystem and most ingested microorganisms fail to colonize because of the lack of an available niche (21). Colonization resistance is, in fact, the reason that we use streptomycin, i.e., to clear the mouse intestine of facultative microorganisms and create an available niche. Streptomycin treatment selectively reduces the facultative microflora; however, the anaerobic population in the large intestine remains largely intact, and large numbers of different species coexist (17). Because maximum *E. coli* colonizing ability requires gluconate metabolism via the Entner-Doudoroff pathway, an incoming microorganism that utilizes gluconate better than *E. coli* could displace it and colonize. In this context, it is of great interest that when gluconate is the carbon source, *Yersinia enterocolitica* makes 4- to 26-fold the amount of heat-stable enterotoxin as it does in the presence of a number of other sugars (1). Perhaps *Y. enterocolitica* overcomes colonization resistance by its ability to utilize gluconate and at the same time uses gluconate as a signal to induce synthesis of virulence factors.

In summary, it appears that *E. coli* F-18 and *E. coli* K-12 utilize the gluconate present in mucus for colonization of the streptomycin-treated mouse large intestine. As a consequence, it appears that the Entner-Doudoroff pathway is important for *E. coli* in the intestine. Whether pathogenic strains of *E. coli* rely on gluconate and the Entner-Doudoroff pathway for virulence is under investigation.

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