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The *Escherichia coli* K-12 *gntP* Gene Allows *E. coli* F-18 To Occupy a Distinct Nutritional Niche in the Streptomycin-Treated Mouse Large Intestine

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***Escherichia coli* F-18 is a human fecal isolate that makes type 1 fimbriae, encoded by the *fim* gene cluster, and is an excellent colonizer of the streptomycin-treated mouse intestine. *E. coli* F-18 *fimA*::*tet*, lacking type 1 fimbriae, was constructed by bacteriophage P1 transduction of the *fim* region of the *E. coli* K-12 strain ORN151, containing the tetracycline resistance gene from Tn*10* inserted in the *fimA* gene, into *E. coli* F-18. *E. coli* F-18 *fimA*::*tet* was found to occupy a distinct niche in the streptomycin-treated mouse intestine when fed in small numbers (10^4 CFU) to mice, along with large numbers (10^{10} CFU) of *E. coli* F-18, as defined by the ability of the *E. coli* F-18 *fimA*::*tet* strain to grow and colonize only 1 order of magnitude below *E. coli* F-18. The same effect was observed when mice already colonized with *E. coli* F-18 were fed small numbers of *E. coli* F-18 *fimA*::*tet*. Experiments which show that the *E. coli* K-12 gene responsible for this effect is not *fim*::*tet* but *gntP*, which maps immediately downstream of the *fim* gene cluster, are presented. *gntP* encodes a high-affinity gluconate permease, suggesting that the distinct niche in the mouse large intestine is defined by the presence of gluconate. The data presented here support the idea that small numbers of an ingested microorganism can colonize the intestine as long as it can utilize an available nutrient better than any of the other resident species can.**

A thick (200- to 400-μm), viscous mucus layer covers the epithelial cells of the mammalian large intestine (1, 11). The mucus layer consists of mucin (a gel-forming glycoprotein), glycoproteins, lipids, proteins, nucleic acids, epithelial cell debris, enzymes, bile, and all the components of gastric juices (1, 25). The mucus layer is thought to exist in a dynamic state in which the mucus is continuously being secreted by goblet cells and simultaneously being degraded by the resident microorganisms (23, 36).

An examination of the mammalian intestine has revealed that several hundred species of microorganisms inhabit this environment (35). The diversity of microorganisms reflects their respective abilities to occupy different ecological niches. It has been demonstrated in experiments in chemostats that two different microorganisms having a preference for the same growth-limiting nutrient cannot coexist (41). That is, one will eventually outcompete and eliminate the other. However, if two different microorganisms utilize different growth-limiting nutrients, they can coexist in a chemostat and maintain stable populations (44, 46). These observations have led to the theory that the mammalian intestine may be thought of as a chemostat with several hundred species in equilibrium, each utilizing a different limiting nutrient better than all the other species (19, 30). The size of any particular population would then be dependent on and proportional to the concentration of the corresponding nutrient. The plethora of substrates present in the intestinal tract arise from ingested and digested food, epithelial cell debris, mucus, and substrates secreted by the microflora. An exception to this nutrient/niche theory was developed by Freter, who used mathematical modeling to demonstrate that two species competing for the same limiting nutrient may occupy the same niche if the less efficient species can adhere to the intestinal mucosa (20). Thus, two classes of ecological niches may exist in the intestine, specific adhesion niches and specific nutrient niches.

Although many species of bacteria found in the mammalian large intestine have been characterized and cataloged, relatively little is known about how they compete with each other nutritionally, i.e., which substrates are available to support growth, which metabolic pathways are used, and which genes are required for the ability to colonize. 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. This phenomenon, predicted by the theories described above, has been noted by several extensive studies (8, 9, 17, 18, 45). For example, when human volunteers were fed with *E. coli* K-12 strains, these strains did not persist in the feces of these individuals (2, 32, 43). Moreover, when healthy human volunteers were fed *E. coli* strains isolated from their own feces, even those strains could not colonize (3).

Despite the phenomenon of colonization resistance, a continuous succession of *E. coli* strains appears to exist in the mammalian intestine. Some strains are present for extended periods from months to years (residents), whereas others

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

Strain or plasmid	Relevent characteristics	Source or reference
Strains		
F-18	Str^r Rif^r	13
F-18 Nal^r	Str^r Rif^r Nal^r	34
F-18 *fimA*::*tet*	Transduction of *fimA*::*tet* from ORN151 to F-18, Str^r Rif^r Tet^r	34
F-18 *fimA*::*npt*	Transduction of *fimA*::*npt* from ORN147 to F-18, Str^r Rif^r Kan^r	This study
F-18 no. 99	Transduction of *fim* region from ORN174 to F-18 *fimA*::*npt*; Str^r Rif^r Tet^r	This study
F-18 no. 167	Transduction of *fim* region from ORN174 to F-18 *fimA*::*npt*; Str^r Rif^r Tet^r	This study
F-18 no. 167 *uxuA*::miniTn*10*::*npt*	miniTn*10*::*npt* into *uxuA* of F-18 no. 167, Str^r Rif^r Tet^r Kan^r	This study
ORN151	*rpsL fimA*::*tet*, Str^r Tet^r	33
ORN147	*rpsL fimA*::*npt*, Str^r Kan^r	33
ORN174	*tet* inserted 400 bp downstream of *fimH*	7
ATM160	λ*pir*, contains pLOF	14, 22
Plasmids		
pRU1	*fimF fimG fimH gntP uxuA uxuB E. coli* K-12 genes cloned into *Bam*HI site of pBR322	37
pRU6	*E. coli* K-12 *uxuA* gene cloned into *Sal*I site of pBR322	37
pPKL133	2.6-kb *Kpn*I-*Eco*RI fragment of pPIL38 containing *gntP* cloned into *Kpn*I-*Eco*RI restricted plasmid pGEM3	28
pGEM3	Amp^r	Promega
pLOF	Suicide vector containing mini Tn*10*::*npt*	14, 22

(transients) can be detected for only several days (38–40). In one study in which the plasmid profiles of different *E. coli* strains in fecal samples obtained from one person were examined during an 11-month period, 53 different electrophoretic types were found, and although most were detected for only a few sampling days, some were present for extended periods (12). In another study, an average of five *E. coli* biotypes were found in the feces of individual humans (4). Thus, diversity exists even among normal commensal *E. coli* strains in the intestine, suggesting the possibility that different *E. coli* strains utilize different growth-limiting nutrients for colonization.

In the present study, we present a situation in which an *E. coli* K-12 *fim*-linked gene allows *E. coli* F-18, a normal human fecal isolate (13), to occupy a distinct niche in the mouse large intestine, as defined by its ability, when ingested in small numbers, to grow and colonize in the presence of large numbers of its parent. Moreover, we show that the *E. coli* K-12 *fim*-linked gene is *gntP*, a recently described gene which encodes a high-affinity gluconate permease (28).

MATERIALS AND METHODS

Bacterial strains and plasmids. All the *E. coli* F-18 and K-12 strains and plasmids used in this study are listed in Table 1.

Bacteriophage P1 transduction. All P1 transductions were performed as previously described (34).

Construction of *E. coli* F-18 strain 167 *uxuA*::miniTn*10*::*npt*. *E. coli* F-18 strain 167 *uxu* A::miniTn*10*::*npt*, which is unable to metabolize glucuronate, was constructed by insertional mutagenesis (14, 22). *E. coli* ATM160, carrying the suicide vector pLOF, was conjugated with *E. coli* F-18 no. 167 (Table 1) in the following manner. Recipient and donor 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, cells on the filter were suspended in 5 ml of 10 mM $MgSO_4$, and 100-μl aliquots of the suspension were plated on Luria agar containing streptomycin (100 μg/ml) and kanamycin sulfate (80 μg/ml). The resulting colonies (approximately 2,000) were then toothpicked onto Davis minimal agar plates containing either glucose (1 mg/ml), glucuronate (2.5 mg/ml), gluconate (2.5 mg/ml), or galacturonate (2.5 mg/ml). One colony grew on glucose, gluconate, and galacturonate but not glucuronate. The strain was sensitive to ampicillin (100 μg/ml), eliminating the possibility that pLOF integrated into the chromosome. When this strain was complemented with pRU6 (containing a functional *uxuA* gene), glucuronate metabolism was restored (Table 2). Plasmid pJVN27 (control vector) did not restore glucuronate metabolism. The strain was designated *E. coli* F-18 no. 167 *uxuA*::miniTn*10*::*npt*.

The *uxuA* gene maps at 98.1 min on the *E. coli* K-12 chromosome (6), downstream of the *fim* gene cluster. To confirm the location of the *uxuA*::miniTn*10*::*npt* mutation, the cotransduction frequency of *fim* and *uxuA*::miniTn*10*::*npt* from *E. coli* F-18 no. 167 *uxuA*::miniTn*10*::*npt* to *E. coli* K-12 was determined. Eighteen *E. coli* K-12 *uxuA*::miniTn*10*::*npt* transductants (kanamycin resistant and unable to utilize glucuronate as the sole carbon source but able to utilize glucose, gluconate, or galacturonate) were tested for the *fim* region of *E. coli* F-18 no. 167 *uxuA*::miniTn*10*::*npt* (marked with *tet*, [Table 1]). As expected for closely linked genes, 12 of the 18 *E. coli* K-12 *uxuA*::miniTn*10*::*npt* transductants (67%) were tetracycline resistant.

DNA techniques. *E. coli* HB101 (10) was transformed with plasmids via electroporation with a gene pulser (Bio-Rad Laboratories, Richmond, Calif.). Plasmids were isolated from transformed *E. coli* HB101 on Promega Magic miniprep columns (Promega Corp., Madison, Wis.) and subsequently electroporated into *E. coli* F-18 and K-12 strains.

Agglutination of *E. coli* F-18 strains by rabbit-specific anti-type 1 fimbria serum. All *E. coli* F-18 strains used in this study were tested for the presence of type 1 fimbriae after growth to stationary phase at 37°C in Luria broth. Aliquots of 100 μl of each culture were added to 10 μl of undiluted rabbit-specific anti-type 1 fimbria serum (34) for 5 min at room temperature, and the mixtures were observed by phase-contrast microscopy (magnification, ×400 for the extent of agglutination). The antiserum was preadsorbed with whole *E. coli fimA*::*tet* to prevent nonspecific binding of antibodies to the test cells.

Mouse colonization experiments. The method used to compare the relative

TABLE 2. Utilization of various carbohydrates by *E. coli* F-18 strains

E. coli strain	Utilization of[a]:			
	Glc	GlcA	GalA	Gnt
F-18	+	+	+	+
F-18 *fimA*::*tet*	+	+	+	+
F-18 *fimA*::*npt*	+	+	+	+
F-18 no. 167	+	+	+	+
F-18 no. 167 *uxuA*::miniTn*10*::*npt*	+	0	+	+
F-18 no. 167 *uxuA*::miniTn*10*::*npt*(pRU1)	+	+	+	+
F-18 no. 167 *uxuA*::miniTn*10*::*npt*(pRU6)	+	+	+	+
F-18(pPKL133)	+	+	+	+
F-18(pGEM3)	+	+	+	+

[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). Davis minimal agar plates were incubated at 30°C for 48 h.

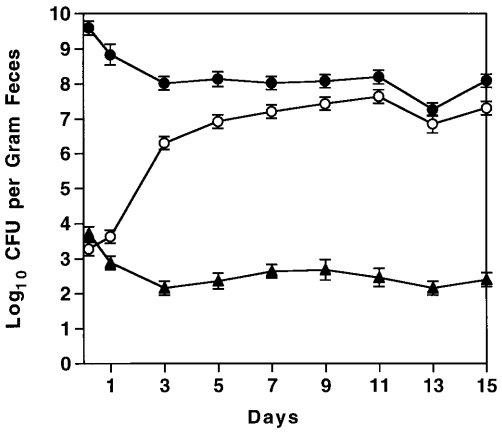

FIG. 1. Colonization of *E. coli* F-18, *E. coli* F-18 *fimA*::*tet*, and *E. coli* F-18 Nal^r. Six streptomycin-treated mice were fed 10^{10} CFU of *E. coli* F-18 (●) and 10^4 CFU each of *E. coli* F-18 *fimA*::*tet* (○) and *E. coli* F-18 Nal^r (▲). On the days indicated, fecal samples were diluted and plated on MacConkey agar containing streptomycin sulfate and either rifampin, rifampin and tetracycline hydrochloride, or rifampin and nalidixic acid to differentiate the three strains. Results are presented as $\log_{10}$ mean CFU per gram of feces ± the standard error (SE) of the $\log_{10}$ mean.

intestinal colonization abilities of streptomycin-resistant *E. coli* strains in mice has been described previously (13, 34). Briefly, three male, individually housed CD-1 mice (5 to 8 weeks of age), fed Charles River Valley rat, mouse, and hamster formula, were given streptomycin-treated (5 g/liter) drinking water for 24 h. Following 18 to 24 h of starvation for food and water, the mice were given 1 ml of 20% (wt/vol) sucrose containing either 10^{10} or 10^4 CFU of Luria broth-grown *E. coli* strains. The strains were given either alone or along with one or two other strains. After the bacterial suspension had been ingested, both the food and streptomycin-treated 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. Cages were changed daily. Fecal samples (no older than 24 h) were homogenized, diluted in 1% tryptone broth, and plated on MacConkey agar (Difco, Detroit, Mich.) 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, 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 performed twice to confirm initial colonization results, and the data from both experiments are included in the figures.

Southern blotting. Southern hybridizations were performed at high stringency with the digoxigenin nonradioactive DNA-labeling and detection kit (Boehringer Mannheim). The *gntP* gene of *E. coli* PC31 (28) was used as the probe.

RESULTS

***E. coli* F-18 *fimA*::*tet* occupies a distinct niche in the mouse large intestine.** The *fim* gene cluster encodes the genes required for the synthesis of type 1 fimbriae (26, 27, 33). *E. coli* F-18 *fimA*::*tet*, unable to make type 1 fimbriae, contains the chromosomal *fim* region from the *E. coli* K-12 strain ORN151 (*fimA*::*tet*) (33). As previously described, when mice were fed 10^{10} CFU each of *E. coli* F-18 and *E. coli* F-18 *fimA*::*tet*, both strains colonized at approximately the same level of about 10^8 CFU/g of feces (34). However, when mice were fed 10^{10} CFU of *E. coli* F-18 and 10^4 CFU each of *E. coli* F-18 *fimA*::*tet* and *E. coli* F-18 Nal^r, *E. coli* F-18 colonized at approximately 10^8 CFU/g of feces, *E. coli* F-18 *fimA*::*tet* colonized at approximately 10^7 CFU/g of feces, and *E. coli* F-18 Nal^r colonized at approximately 10^2 CFU/g of feces (Fig. 1). Thus, *E. coli* F-18 and *E. coli* F-18 Nal^r were equally good colonizers, since they maintained a ratio of 10^6:1 throughout the entire 15 days. However, *E. coli* F-18 *fimA*::*tet* appeared to grow in and occupy a distinct niche from which *E. coli* F-18 Nal^r was excluded. When the experiment was performed in the opposite direction, i.e., when 10^{10} CFU of *E. coli* F-18 *fimA*::*tet* and 10^4 CFU of *E. coli* F-18 Nal^r were fed to mice, *E. coli* F-18 *fimA*::*tet* colonized at a level of about 10^8 CFU/g of feces whereas *E. coli* F-18 Nal^r colonized at about 10^2 CFU/g of feces (results not shown). Therefore, *E. coli* F-18 Nal^r was not able to occupy the distinct niche available to *E. coli* F-18 *fimA*::*tet*. It should be noted that when occupying the distinct niche, *E. coli* F-18 *fimA*::*tet* was not only found in feces but was also found in ileal, cecal, and colonic mucus (not shown).

E. coli F-18 *fimA*::*tet* was also able to grow in and occupy the distinct niche in mice that had been colonized with *E. coli* F-18 for 15 days. That is, when mice colonized with *E. coli* F-18 at a level of about 10^8 CFU/g of feces were challenged with 10^4 CFU each of *E. coli* F-18 *fimA*::*tet* and *E. coli* F-18 Nal^r, *E. coli* F-18 *fimA*::*tet* grew and colonized at a level of about 10^7 CFU/g of feces whereas *E. coli* F-18 Nal^r decreased to $<10^2$ CFU/g of feces (Fig. 2).

In control experiments, it was found that *E. coli* F-18 *fimA*::*npt*, which does not make type 1 fimbriae because it contains the *fim* chromosomal region from *E. coli* K-12 ORN147 (*fimA*::*npt*), and two randomly selected *E. coli* F-18 P1 transductants (no. 99 and 167) which contain the *fim*$^+$ gene cluster of *E. coli* ORN152 and which make type 1 fimbriae, were all able to occupy the distinct niche (data not shown). Therefore, the ability of *E. coli* F-18 *fim* transductants to occupy the distinct niche was not associated with either tetracycline resistance or the ability to make type 1 fimbriae but appeared to be due to a *fim*-linked gene present in the ORN strains that was either absent or nonfunctional in *E. coli* F-18. Since the ORN strains were derived from *E. coli* K-12, we will refer to the gene as the K-12 *fim*-linked gene.

A glucuronate mutant can occupy the distinct site. D-Glucuronate is a major constituent of hyaluronic acid, which is plentiful in mammalian tissues and makes up approximately 0.6% of the dry weight of mouse cecal mucus (28a). The *uxu* gene cluster maps downstream of the *fim* gene cluster at 98 min on the *E. coli* K-12 chromosome and consists of three genes, *uxuA*, which encodes D-mannonate dehydratase (EC 4.2.1.8), *uxuB*, which encodes D-mannonate oxidoreductase (EC 1.1.1.57), and *uxuR*, which encodes a negative repressor of *uxuA* and *uxuB* (48). To determine whether the K-12 *fim*-

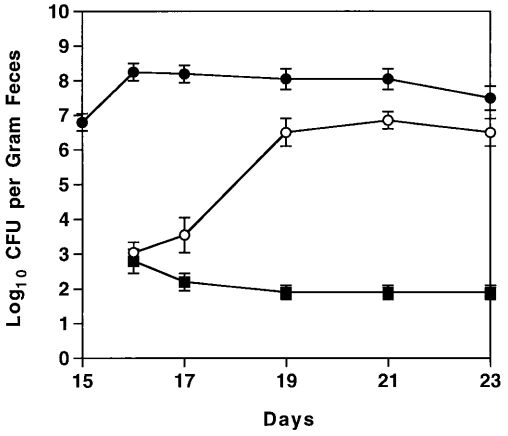

FIG. 2. Colonization of *E. coli* F-18 *fimA*::*tet* and *E. coli* F-18 Nal^r in mice precolonized with *E. coli* F-18. Six streptomycin-treated mice were fed 10^{10} CFU of *E. coli* F-18 (●) and 15 days later were fed 10^4 CFU each of *E. coli* F-18 *fimA*::*tet* (○) and *E. coli* F-18 Nal^r (■). On the days indicated, fecal samples were diluted and plated on MacConkey agar containing streptomycin sulfate and either rifampin, rifampin and tetracycline hydrochloride, or rifampin and nalidixic acid to differentiate the three strains. Results are presented as $\log_{10}$ mean CFU per gram of feces ± SE of the $\log_{10}$ mean.

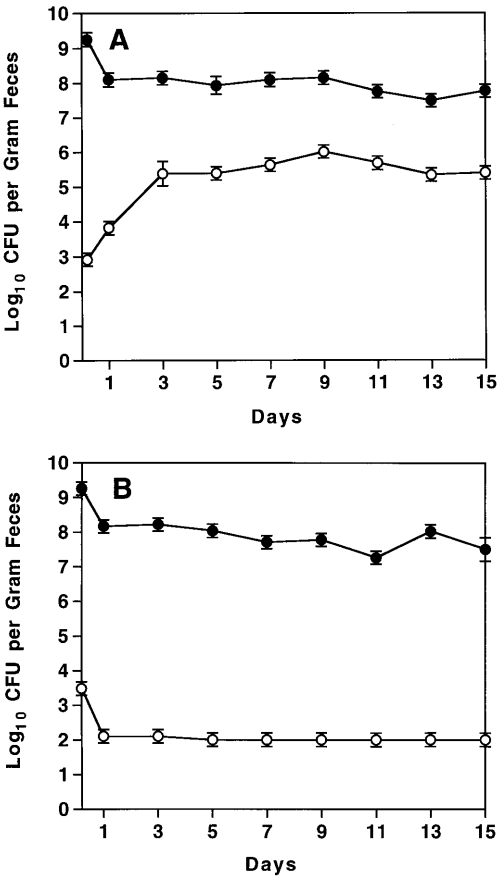

FIG. 3. Colonization of *E. coli* F-18 Nal^r, *E. coli* F-18(pRU1), and *E. coli* F-18(pRU6). Six streptomycin-treated mice were fed 10^{10} CFU of *E. coli* F-18 Nal^r (●) and 10^4 CFU of *E. coli* F-18(pRU1) (○) (A) and 10^{10} CFU of *E. coli* F-18 Nal^r (●) and 10^4 CFU of *E. coli* F-18(pRU6) (○) (B). On the days indicated, fecal samples were diluted and plated on MacConkey agar plates containing streptomycin sulfate and either nalidixic acid or ampicillin. Results are presented as $\log_{10}$ mean CFU per gram of feces ± SE of the $\log_{10}$ mean.

linked gene was involved in glucuronate metabolism, we constructed *E. coli* F-18 no. 167 *uxuA*::miniTn*10*::*npt*, which makes type 1 fimbriae because it contains the wild-type K-12 *fim* gene cluster but is unable to metabolize glucuronate (Tables 1 and 2). Streptomycin-treated mice were fed 10^{10} CFU of *E. coli* F-18 Nal^r and 10^4 CFU of *E. coli* F-18 no. 167 *uxuA*::miniTn*10*::*npt*. *E. coli* F-18 Nal^r colonized at about 10^8 CFU/g of feces, and *E. coli* F-18 no. 167 *uxuA*::miniTn*10*::*npt* colonized the distinct niche at between 10^6 and 10^7 CFU/g of feces (data not shown). Therefore, the inability to metabolize glucuronate did not abolish the ability to colonize the distinct site.

The K-12 *fim*-linked gene is *gntP*. pRU1 contains *fimF*, *fimG*, *fimH*, *gntP*, *uxuA*, and *uxuB* (see Fig. 5). When mice were challenged with 10^{10} CFU of *E. coli* F-18 Nal^r and 10^4 CFU of *E. coli* F-18(pRU1), *E. coli* F-18 Nal^r colonized at about 10^8 CFU/g of feces and *E. coli* F-18(pRU1) colonized at approximately 10^6 CFU/g of feces (Fig. 3A), indicating that plasmid pRU1 contains the K-12 *fim*-linked gene. The only nonvector gene that pRU6 contains is a functional *uxuA* gene (36), which restored glucuronate metabolism to *uxuA* mutants (Table 2). When mice were fed 10^{10} CFU of *E. coli* F-18 Nal^r and 10^4 CFU of *E. coli* F-18(pRU6), *E. coli* F-18 Nal^r colonized at about 10^8 CFU/g of feces while *E. coli* F-18(pRU6) was eliminated from the intestine (i.e., below the detection limit of 10^2 CFU/g of feces) (Fig. 3B).

Since the K-12 *uxu* genes did not appear to be involved in *E. coli* F-18 occupying the distinct niche and it was unlikely that *fimF*, *fimG*, and *fimH* were involved, we tested the possibility that *gntP*, the only other K-12 gene on pRU1, was the K-12 *fim*-linked gene (see Fig. 5). The functional K-12 *gntP* gene is the only non-pGEM3 gene on pPKL133 (see Fig. 5). It encodes a high-affinity gluconate permease (28). When mice were challenged with 10^{10} CFU of *E. coli* F-18 and 10^4 CFU of *E. coli* F-18(pPKL133), *E. coli* F-18 colonized at about 10^8 CFU/g of feces and *E. coli* F-18(pPKL133) colonized at about 10^7 CFU/g of feces (Fig. 4A); i.e., pPKL133 allowed *E. coli* F-18 to occupy the distinct niche. In contrast, the vector control pGEM3 did not allow *E. coli* F-18 to occupy the distinct niche (Fig. 4B). These data therefore suggest that the K-12 *fim*-linked gene that allows *E. coli* F-18 to occupy the distinct niche is *gntP*.

***E. coli* F-18 does not contain *gntP*.** Southern blotting experiments were performed under high-stringency conditions to determine whether *E. coli* F-18 contains *gntP*. DNA isolated from both *E. coli* PC31, a K-12 strain, and *E. coli* SK22, a human commensal strain, reacted very strongly with the *gntP* probe (1.2-kb *Nsi*I fragment [Fig. 5]), yielding the predicted 6.7-kb fragment released by *Eco*RV treatment (Fig. 5, lanes 1 and 7), the predicted 5.5-kb fragment released by *Eco*RI treatment (lanes 2 and 8), and the predicted 1.8-kb fragment con-

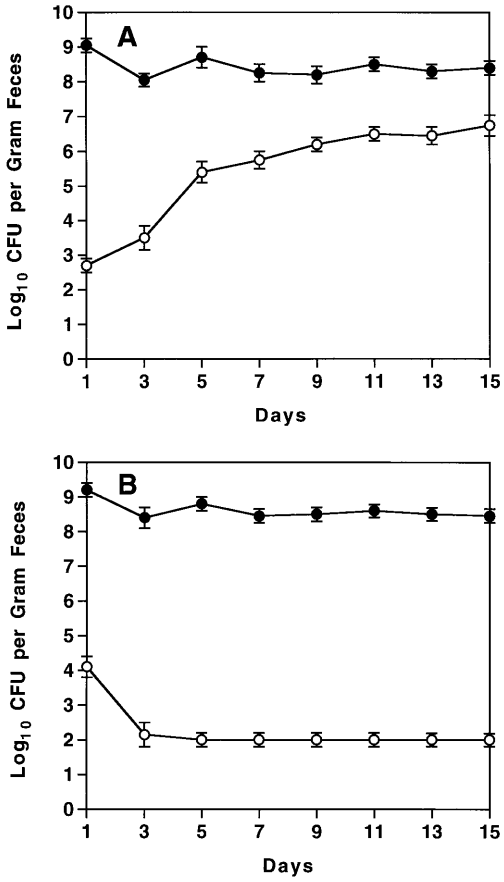

FIG. 4. Colonization of *E. coli* F-18 Nal^r, *E. coli* F-18(pPKL133), and *E. coli* F-18(pGEM3). Six streptomycin-treated mice were fed 10^{10} CFU of *E. coli* F-18 Nal^r (●) and 10^4 CFU of *E. coli* F-18(pPKL133) (○) (A) and 10^{10} CFU of *E. coli* F-18 Nal^r (●) and 10^4 CFU of *E. coli* F-18(pGEM3) (○) (B). On the days indicated, fecal samples were diluted and plated on MacConkey agar plates containing streptomycin sulfate and either nalidixic acid or ampicillin. Results are presented as $\log_{10}$ mean CFU per gram of feces ± SE of the $\log_{10}$ mean.

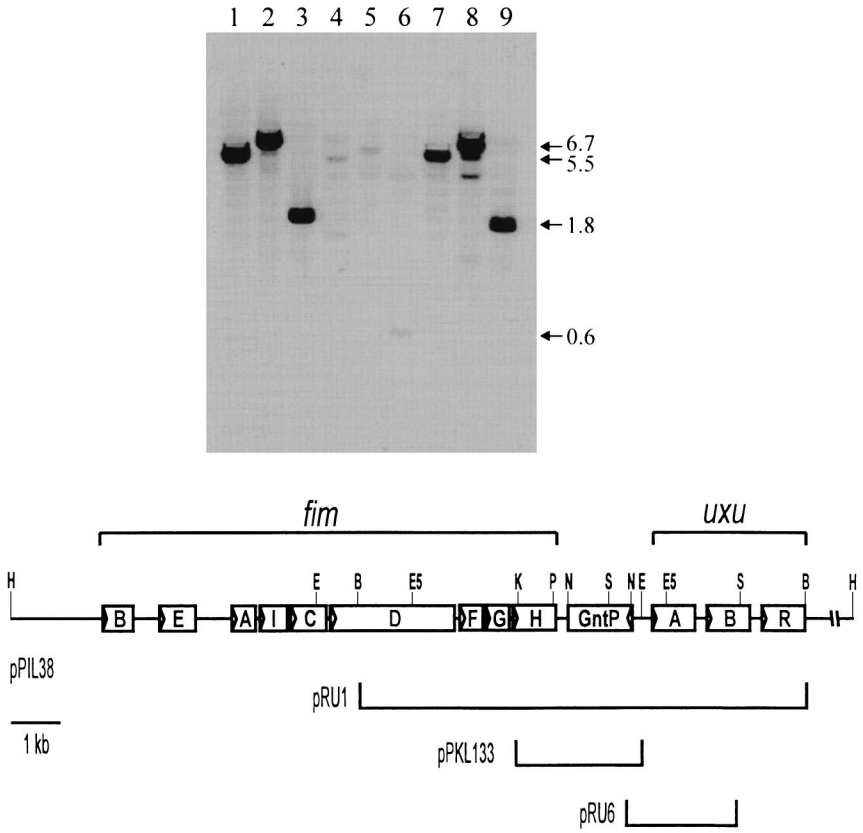

FIG. 5. Southern blotting and *E. coli* K-12 genes located in pRU1, pRU6, and pPKL133. Equal amounts of genomic DNA isolated from *E. coli* PC31, F-18, and SK22 were subjected to restriction enzymes and Southern hybridization as follows: lanes 1 to 3, *E. coli* PC31 DNA; lanes 4 to 6, *E. coli* F-18 DNA; lanes 7 to 9, *E. coli* SK22 DNA. Lanes 1, 4, and 7 contain DNA cut with *Eco*RV; lanes 2, 5, and 8 contain DNA cut with *Eco*RI; lanes 3, 6, and 9 contain DNA cut with *Eco*RI and *Pst*I. The digoxigenin-labeled *Nsi*I fragment of *gntP* was used as the probe. Abbreviations: B, *Bam*HI; E, *Eco*RI; E5, *Eco*RV; H, *Hin*dIII; K, *Kpn*I; N, *Nsi*I; P, *Pst*I; S, *Sal*I.

taining predominantly *gntP* DNA released by *Eco*RI and *Pst*I treatment (lanes 3 and 9). In contrast, *E. coli* F-18 DNA reacted very weakly with both the 6.7- and 5.5-kb fragments and, instead of reacting with the expected 1.8-kb *gntP* fragment, reacted very weakly with a 0.6-kb *Eco*RI-*Pst*I fragment (lanes 4 to 6). These results indicate that *E. coli* F-18 does not contain a functional *gntP* gene. In similar Southern blotting experiments, *E. coli* F-18 *fimA*::*tet* was found to contain the *gntP* gene (results not shown), suggesting that *gntP* is also responsible for its ability to colonize the distinct niche.

DISCUSSION

In a previous report, we suggested that type 1 fimbriae prevent *E. coli* F-18 from occupying the distinct niche described here (29); however, in that study, *fimA*::*tet* had been bacteriophage P1 transduced from an *E. coli* K-12 strain into *E. coli* F-18 and we had not eliminated the possibility that the gene responsible was not the defective *E. coli* K-12 *fimA*::*tet* gene but a K-12 *fim*-linked gene. Here, we show that the effect is due to the *E. coli* K-12 *gntP* gene, which is immediately downstream of *fimH* in *E. coli* K-12 (28) and is not present in *E. coli* F-18 (Fig. 5). Since *gntP* encodes a gluconate permease, the distinct niche is most likely to be a nutritional niche defined by the presence of gluconate.

As stated above, 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 a lack of an available niche (30). Colonization resistance is, in fact, the reason that we used streptomycin, i.e., to clear the mouse intestine of facultative microorganisms and create an available niche for our *E. coli* strains. 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 (21). Since *E. coli* K-12 fails to colonize the intestines of conventional mice (20), streptomycin treatment may be eliminating a microorganism that either utilizes gluconate better than *E. coli* K-12 does or kills it.

Gluconate is metabolized in *E. coli* K-12 via the Entner-Doudoroff pathway and to a lesser extent via the pentose phosphate pathway (15, 47). *E. coli* K-12 contains three gluconate permeases other than GntP, each encoded by a different gene. The GntI system of genes, which maps at 75 min on the chromosome, contains *gntT* and *gntU*, encoding high- and low-affinity gluconate transport systems, respectively, and *gntK*, encoding a gluconate kinase (24). The GntII system, located at 96 min, contains *gntS*, encoding a second high-affinity gluconate transport system, and *gntV*, encoding a thermosensitive gluconate kinase (5). The GntI system is specifically induced by gluconate and is regulated by *gntR*, a repressor that also maps at 75 min (48). GntR also represses the Entner-Douderoff pathway but does not regulate the genes of the GntII system. Genes regulating the GntII system have not been identified (24).

E. coli F-18 does not contain the *gntP* gene (Fig. 5) but does metabolize gluconate (Table 2). This suggests that *E. coli* F-18 has one or more of the gluconate permease genes discussed above but that these genes are not expressed as well as *gntP* in the mouse intestine or that the *E. coli* F-18 gluconate permease(s) is not as effective as GntP in transporting gluconate in the intestine.

The *E. coli* K-12 *gntP* gene is monocistronic and appears to be under catabolite repression; i.e., it contains an excellent catabolite activator protein-binding site and is repressed by glucose (28). The GntP protein is a high-affinity inner membrane gluconate permease and has a much higher affinity for gluconate than for a number of other sugars, including glucuronate (28). Interestingly, the expression of *gntP* is constitutive in exponentially growing cells in the absence of gluconate or a catabolite repressing carbon source (28). Moreover, *gntP* is repressed by high concentrations of gluconate (28).

The fact that *gntP* encodes a high-affinity constitutive gluconate transport system in the absence of high gluconate concentrations suggests that it is ideally suited to function in an environment containing growth-limiting amounts of gluconate. The mouse large intestine is most likely to be such an environment. A primary source of gluconate in the intestine is likely to be 6-phosphogluconate, which is a key intermediate in the mammalian and bacterial pentose phosphate pathways (16, 30). We suggest the possibility that as dead epithelial cells are sloughed from intestinal villus tips into the mucus layer, 6-phosphogluconate is released into the mucus along with dead epithelial cell contents. The action of presumptive 6-phosphogluconate-specific phosphatases present in mucus, of either epithelial cell or bacterial cell origin, could generate gluconate. A second gluconate source in the large intestine could of course be undigested dietary gluconate.

Since *gntP* is repressed by high concentrations of gluconate (28), we suggest that gluconate must be present in low concentrations in the streptomycin-treated mouse large intestine. Moreover, we suggest that the *E. coli* K-12 *gntP* gene, either in the chromosome of *E. coli* F-18 or in a plasmid, allows *E. coli* F-18 to utilize growth-limiting amounts of gluconate not only better than *E. coli* F-18 but also better than any of the myriad of other species also present in the streptomycin-treated mouse large intestine (21). In addition, since *E. coli* F-18 colonizes at about 10^8 CFU/g of feces and *E. coli* F-18 carrying the K-12 *gntP* colonizes about an order of magnitude lower when fed in small numbers to mice, *E. coli* F-18 must be using something other than gluconate as its major carbon source in the large intestine.

Finally, the data presented here suggest that at least with respect to *E. coli* strains, the nutrient/niche theory is correct. That is, in the presence of an established *E. coli* strain, small numbers of another ingested *E. coli* strain will grow and colonize as long as it can utilize a growth-limiting nutrient better than the first *E. coli* strain and all the other species present in the intestine.

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