

Glycogen and Maltose Utilization by *Escherichia coli* O157:H7 in the Mouse Intestine[∇]

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Received 23 January 2008/Returned for modification 23 February 2008/Accepted 7 March 2008

Mutant screens and transcriptome studies led us to consider whether the metabolism of glucose polymers, i.e., maltose, maltodextrin, and glycogen, is important for *Escherichia coli* colonization of the intestine. By using the streptomycin-treated mouse model, we found that catabolism of the disaccharide maltose provides a competitive advantage in vivo to pathogenic *E. coli* O157:H7 and commensal *E. coli* K-12, whereas degradation of exogenous forms of the more complex glucose polymer, maltodextrin, does not. The endogenous glucose polymer, glycogen, appears to play an important role in colonization, since mutants that are unable to synthesize or degrade glycogen have significant colonization defects. In support of the hypothesis that *E. coli* relies on internal carbon stores to maintain colonization during periods of famine, we found that by providing a constant supply of a readily metabolized sugar, i.e., gluconate, in the animal's drinking water, the competitive disadvantage of *E. coli* glycogen metabolism mutants is rescued. The results suggest that glycogen storage may be widespread in enteric bacteria because it is necessary for maintaining rapid growth in the intestine, where there is intense competition for resources and occasional famine. An important implication of this study is that the sugars used by *E. coli* are present in limited quantities in the intestine, making endogenous carbon stores valuable. Thus, there may be merit to combating enteric infections by using probiotics or prebiotics to manipulate the intestinal microbiota in such a way as to limit the availability of sugars preferred by *E. coli* O157:H7 and perhaps other pathogens.

In nature, the nutrients that support bacterial growth are rarely available continuously and are almost always present in limiting amounts, leading to the idea that bacterial existence is one of feast or famine (24). Others have proposed the existence of an in-between state, namely hunger, which is defined by expression of carbon-scavenging systems (18). In the mammalian gastrointestinal tract (9), despite growing in a mucus layer rich in carbohydrates, lipids, and proteins (27), *Escherichia coli* apparently leads a scavenging lifestyle, using up to seven different sugars to support its colonization (7, 16). It is likely that the availability of one or more of its preferred nutrients in the intestine is limited and variable. Thus, for *E. coli* to cope with life in a nutrient-limiting environment where it is hungry or occasionally starving, we hypothesize that successful competition and persistence in the intestine requires intracellular energy stores. Glycogen is the primary carbon and energy storage molecule for enteric bacteria, including *E. coli* (5).

The discovery of glycogen in *E. coli* is credited to Cedergren and Holme (6). In an early review of bacterial cell composition, it was Neidhardt who first suggested that glycogen degradation might be linked to energy metabolism (34). It is now well established that glycogen is the fundamental carbon and energy storage compound for many bacteria and plays a major role in long-term survival of the cell. Glycogen is synthesized

during times when carbon is abundant but other nutrients are limiting (36). GlgA, glycogen synthase, is responsible for the transfer of the glucosyl unit from ADP-glucose to the growing glycogen polymer to form a new α -1,4-glucosidic linkage (37). GlgB is the branching enzyme that catalyzes formation of α -1,6-glucosidic linkages. GlgC, glucose-1-phosphate adenylyltransferase, forms ADP-glucose from glucose-1-phosphate and ATP. GlgS is required for glycogen synthesis, its overproduction stimulates glycogen synthesis, and it is induced upon entry into stationary in an RpoS-dependent manner (21). However, GlgS has no defined role in glycogen synthesis, although its crystal structure suggests involvement in protein-protein interactions (25). Glycogen degradation occurs when carbon sources become limiting (13). GlgP, glycogen phosphorylase, catalyzes glycogen breakdown by removing glucose units from the polysaccharide. GlgX, glycogen debranching enzyme, hydrolyzes α -1,6-glucosidic linkages in limit dextrins generated by GlgP (12).

In salmonellae, the role of glycogen metabolism in colonization and virulence is controversial, with one study suggesting a connection between glycogen storage and virulence (4) and another showing a minor role, at best (30). The contributions of glycogen synthesis and degradation to animal colonization have not been tested for *E. coli*. In support of the hypothesis that glycogen metabolism is important for colonization, we previously found that one of the genes involved in glycogen metabolism, *glgS*, is highly induced in *E. coli* MG1655 when growing on mucus, conditions that are thought to mimic nutrient availability in the intestine (7).

Since glycogen breakdown involves maltose and maltodextrins

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[∇] Published ahead of print on 17 March 2008.

as intermediates, we also thought to examine whether catabolism of exogenous maltose and maltodextrins supports colonization. This possibility seemed reasonable since maltose metabolism genes are also induced in *E. coli* MG1655 when growing on mucus (7). Maltose consists of two glucose molecules joined by an α -1,4 linkage; maltodextrins are longer glucose polymers. These sugars are most commonly derived from starch and glycogen. At least nine genes encode proteins that are involved in the utilization of maltose and maltodextrins in *E. coli* (29). The maltose-inducible porin for maltodextrin transport into the periplasm is encoded by *lamB* (46). The maltose/maltodextrin transporter is a high-affinity ABC system consisting of a periplasmic maltose-binding protein, encoded by *malE*, and a transport complex encoded by *malF*, *malG*, and *malK* (5). *malQ* (amylomaltase) and *malP* (maltodextrin phosphorylase) encode enzymes for maltose and maltodextrin catabolism, respectively. *MalQ* uses maltodextrins as maltose acceptors in a reaction that releases glucose, which enters glycolysis after being phosphorylated by glucokinase (5). *MalP* cleaves glucosyl residues from the nonreducing end of maltodextrins, forming α -glucose-1-phosphate, which enters glycolysis after conversion to glucose 6-phosphate by phosphoglucosmutase (5). Mutants lacking *MalQ* cannot grow on maltose, whereas *malP* mutants can grow on maltose but not on maltodextrins (14).

We previously identified several monosaccharides utilized during colonization of the mouse intestine by pathogenic *E. coli* EDL933 (16) and commensal *E. coli* MG1655 (7). Here, we explore the possibility that complex sugars, i.e., maltose, maltodextrin, and glycogen, support intestinal colonization. The results demonstrate that maltose, but not maltodextrin, is important for colonization of both pathogenic and commensal *E. coli* strains. In addition, glycogen synthesis and glycogen degradation are important for efficient intestinal colonization, suggesting a hunger or famine lifestyle for *E. coli* in the mouse intestine. The results increase our understanding of nutrient utilization in vivo and provide new insights into host-pathogen interactions.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in the present study were derived from *E. coli* MG1655 Str^r (streptomycin resistant), a K-12 strain (32), and *E. coli* EDL933 Str^r, a streptomycin-resistant derivative of the prototypical O157:H7 strain (31). The bacterial strains and plasmids used in the present study are listed in Table 1.

Mutant constructions. Null alleles were constructed by using the allelic replacement method of Datsenko and Wanner (11), as described previously (7, 16), such that target genes were deleted and replaced with kanamycin or chloramphenicol resistance cassettes, which served as selectable markers in mouse colonization assays, as described below. The null allele strains are identified in the text by the genes that were deleted; single gene deletions began with the start codon and ended with the stop codon, and consecutive gene deletions began with the start codon of the first gene deleted and ended with the stop codon of the last gene deleted. Strains containing multiple mutations were constructed by sequential allelic replacement; the first inserted cassette was removed with FLP recombinase (11), followed by subsequent allelic replacement(s) and removal of the insertion as necessary, leaving the selectable marker in the last mutation made. Mutant strains were verified by phenotype analysis and DNA sequencing.

Phenotypic analysis. To test for the ability to use sole carbon and energy sources, mutants were grown overnight in MOPS [3-(*N*-morpholino) propanesulfonic acid] defined medium (35) containing 0.2% maltose or maltodextrin (maltopentaose to maltoheptaose). Maltose and maltodextrin sensitivity were evaluated by growth on Luria broth containing 0.2% carbohydrate. Cell growth was monitored spectrophotometrically at 600 nm (OD₆₀₀). To test for the ability to synthesize glycogen, strains were grown overnight on Luria plates containing 2% glucose and stained with iodine vapor (14). In addition, the mutants used in the present study were phenotyped for carbon source oxidation by using Biolog

TABLE 1. Strains and plasmids used in this study^a

Strain or plasmid ^a	Genotype ^b	Source or reference
Strains		
MG1655	Wild-type (CGSC 7740)	CGSC ^c
MG1655 Str ^r	Spontaneous Str ^r	32
MG1655 Str ^r Nal ^f	Spontaneous Nal ^f	32
EDL933	Wild-type O157:H7	Alison O'Brien
EDL933 Str ^r	Spontaneous Str ^r	31
EDL933 Str ^r Nal ^f	Spontaneous Nal ^f	31
Derivatives of strain MG1655 Str^r		
<i>glgA</i>	$\Delta glgA::cat$	This study
<i>glgP</i>	$\Delta glgP::cat$	This study
<i>glgP malP</i>	$\Delta glgP \Delta malP::cat$	This study
<i>glgS</i>	$\Delta glgS::cat$	This study
<i>lamB</i>	$\Delta lamB::cat$	This study
<i>malEFG</i>	$\Delta(malE-malG)::cat$	This study
<i>malPQ</i>	$\Delta(malP-malQ)::cat$	This study
<i>malX</i>	$\Delta malX::cat$	This study
Derivatives of strain EDL933 Str^r		
<i>glgA</i>	$\Delta glgA::cat$	This study
<i>glgP</i>	$\Delta glgP::cat$	This study
<i>glgP malP</i>	$\Delta glgP \Delta malP::cat$	This study
<i>glgS</i>	$\Delta glgS::cat$	This study
<i>lamB</i>	$\Delta lamB::cat$	This study
<i>malEFG</i>	$\Delta(malE-malG)::cat$	This study
<i>malEFG malX</i>	$\Delta(malE-malG) \Delta malX::cat$	This study
<i>malP</i>	$\Delta malP::cat$	This study
<i>malPQ</i>	$\Delta(malP-malQ)::cat$	This study
<i>malQ</i>	$\Delta malQ::cat$	This study
<i>malX</i>	$\Delta malX::cat$	This study
<i>ppsA pckA</i>	$\Delta ppsA \Delta pckA::cat$	31
Plasmids		
pKD3	<i>bla cat</i>	11
pKD4	<i>bla kan</i>	11
pKD46	T ^s Ap ^r , P _{araC} - λ -red recombinase	11
pCP20	T ^s Ap ^r , FLP recombinase	11
pTag-mini-Tn5Km2	Ap ^r Kn ^r ; mini-Tn5 transposon delivery plasmid	22

^a For simplicity, strains are named by strain and mutation(s).

^b Nal^f, nalidixic acid resistance; Ap^r, ampicillin resistance; Str^r, streptomycin resistance; Kn^r, kanamycin resistance; T^s, temperature-sensitive replication.

^c *E. coli* Genetic Stock Culture Collection, Yale University.

GN2 (carbon nutrition) plates according to the manufacturer's instructions. Briefly, the mutants were grown overnight on tryptic soy agar, washed in Biolog inoculating fluid (gellan gum, 0.2 g/liter; NaCl, 4.0 g/liter; Pluronic F-68, 0.3 g/liter; 2.5 mM thioglycolate), and the plates were inoculated with 150 ml per well of a cell suspension with an OD₆₀₀ of 0.2 to 0.3. The cells were incubated in Biolog plates for 24 h at 37°C in an Omnilog system, and the amount of reduced tetrazolium violet dye in each well was quantified every 15 min. The amount of dye reduced corresponds to the extent of carbon source oxidized. Dye reduction was plotted versus time, and the area under each resulting kinetic curve was used as an overall measure of the cells' ability to utilize each carbon source.

Mouse colonization experiments. The streptomycin-treated mouse model has been used extensively to study colonization of the mouse large intestine by *E. coli* and *Salmonella enterica* serovar Typhimurium (8, 9, 27). Briefly, three 6-week-old CD-1 male mice were given drinking water containing streptomycin sulfate (5 g/liter) for 24 h to selectively remove the existing resident facultative microbiota and then starved for food and water for 18 to 24 h. Unless otherwise indicated, the mice were then fed approximately 10⁵ CFU of both wild-type and mutant strains in 1 ml of 20% sucrose. The wild-type strains were streptomycin resistant

TABLE 2. Competitive colonization between maltose/glycogen mutants and wild-type *E. coli* strains^a

Respiratory enzyme	Mutation	Avg (log ₁₀ CFU/g of feces) ± SEM for:			
		<i>E. coli</i> EDL933 at:		<i>E. coli</i> MG1655 at:	
		Day 1	Day 15	Day 1	Day 15
Maltose transport	<i>malEFG</i>	0.4 ± 0.1	3.3 ± 0.2	0.0 ± 0.1	0.4 ± 0.3
Maltose PTS system	<i>malX</i>	0.4 ± 0.1	0.2 ± 0.2	0.6 ± 0.2	0.6 ± 0.1
Maltose transport and PTS	<i>malEFG malX</i>	0.2 ± 0.1	2.3 ± 0.2	0.6 ± 0.2	2.2 ± 0.2
Maltodextrin phosphorylase and amyloamylase	<i>malPQ</i>	0.8 ± 0.1	3.7 ± 0.1	0.4 ± 0.2	3.5 ± 0.4
Maltodextrin phosphorylase	<i>malP</i>	0.3 ± 0.1	1.4 ± 0.5 ^b	ND ^c	ND
Amyloamylase	<i>malQ</i>	0.3 ± 0.1	3.3 ± 0.4	ND	ND
Maltoporin	<i>lamB</i>	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	0.1 ± 0.2
Glycogen phosphorylase	<i>glgP</i>	0.3 ± 0.1	2.4 ± 0.2	0.5 ± 0.2	3.2 ± 0.1
Glycogen phosphorylase and maltodextrin phosphorylase	<i>glgP malP</i>	0.3 ± 0.2	2.5 ± 0.2	0.4 ± 0.1	2.5 ± 0.2
Glycogen synthase	<i>glgA</i>	0.2 ± 0.1	2.3 ± 0.2	0.2 ± 0.1	2.9 ± 0.2
Glycogen synthesis	<i>glgS</i>	0.2 ± 0.1	1.9 ± 0.1	0.4 ± 0.2	1.6 ± 0.2

^a Mice were fed 10⁵ CFU each of a mutant and its wild-type parent. Mice were transferred to fresh cages every day, and feces samples no older than 24 h were assayed every other day for 15 days. At each time point, for each mouse the log₁₀ CFU/g of feces for the mutant was subtracted from the log₁₀ CFU/g of feces for the wild type. The average ± the standard error of the mean of day 1 and day 15 data from six mice is shown. All values shown in boldface are statistically significant (*P* < 0.005 [Student *t* test]).

^b *P* < 0.08.

^c ND, not determined.

and nalidixic acid resistant (Str^r Nal^r) (31); Nal^r was used to distinguish the wild-type (reference strain) from the null allele mutants in fecal plate counts. After the bacterial suspension was ingested, food and streptomycin-water (5 g/liter) were restored, and fecal plate counts were determined at 5 h, 24 h, and on every other day thereafter for 15 days. Fecal samples were homogenized and diluted in 1% tryptone broth and plated on MacConkey agar either containing streptomycin (100 µg/ml) and nalidixic acid (50 µg/ml) to count the wild type or containing streptomycin and kanamycin (40 µg/ml) or chloramphenicol (30 µg/ml) to count the null allele mutants. Each colonization experiment was repeated, on separate occasions, and averaged for six (or more) mice, such that the presented values are the log₁₀ mean number of CFU per gram of feces ± the standard error for each strain. In all experiments, a difference between two strains of ≥10-fold CFU/g of feces was statistically significant, i.e., *P* < 0.05 as determined by the Student *t* test (two-tailed with unequal variance). The limit of detection in fecal plate counts was 10² CFU/g of feces.

Signature-tagged mutagenesis. A signature-tagged mutant (STM) library of *E. coli* EDL933 Str^r *ppsA pckA* was constructed as previously described (43). A total of 1,440 mutants were arrayed in 30 pools each containing 48 uniquely tagged mutants with mini-Tn5Km2 insertions. The mutant library was screened in streptomycin treated mice (as described above) in competition with *E. coli* MG1655 Str^r Nal^r. Each pool of mutants was grown in Luria broth supplemented with kanamycin in a microtiter plate at 37°C overnight. The mutants from each microtiter plate were pooled, diluted, and mixed with a 10-fold-concentrated overnight culture of *E. coli* MG1655 Str^r Nal^r in 20% sucrose to obtain an inoculum containing approximately 10⁷ CFU of signature-tagged *E. coli* EDL933 Str^r *ppsA pckA* mutants/ml and 10¹⁰ CFU of the *E. coli* MG1655 Str^r Nal^r strain/ml. A 100-µl portion of inoculum was fed to two mice. Signature-tagged *E. coli* EDL933 Str^r *ppsA pckA* mutants unable to persist in the intestine 3 days after inoculation were identified by PCR and hybridization analysis as previously described (43). The competition experiment for colonization was repeated for selected mutants from the screening. The locations of the mini-Tn5Km2 insertions were determined by DNA sequence analysis, as described previously (3).

RESULTS

Colonization assays. Of the animal models available for measuring the relative fitness of two bacterial strains for intestinal colonization, the streptomycin-treated mouse is preferred for the following reasons (for a review of intestinal colonization models, see reference 3). Bioreactors (i.e., intestinal simulators) have proven useful for analyzing specific strain interactions but, in addition to their failure to mimic intestinal

anatomy, it remains unclear what growth medium will duplicate the complex nutritional makeup of the intestinal contents. Conventional mice are resistant to experimental colonization. The gnotobiotic mouse model is a powerful tool for comparing a finite number of strains in direct competition, e.g., how two strains adapt their substrate utilization in response to one another but, in addition to being expensive and time-consuming, this model lacks the diversity of background functions provided by the “normal microbiota,” and gnotobiotic mice exhibit physiological and anatomical differences from conventional animals. Streptomycin treatment selectively removes facultative anaerobes while leaving the anaerobic microbiota essentially intact, i.e., at pretreatment population size; this opens a previously unavailable niche, which can then be colonized by experimentally introduced microorganisms such as *E. coli* (23). In this model, streptomycin-treated mice are fed streptomycin resistant strains of bacteria. Usually, these are two isogenic strains that differ only in a single characteristic, i.e., a mutant and its wild-type parent. If one of these strains possesses even a slight advantage over the other, the conditions of the mouse large intestine select for that strain and within a few days, it becomes dominant. If neither strain possesses an advantage, the two strains cocolonize and are maintained at approximately equal levels. Since the population of a strain in mouse feces is a reflection of its number in the mouse large intestine, fecal counts are used to judge the relative colonizing abilities of *E. coli* strains. The colonization data shown in Table 2 are given for day 1 (initiation) and day 15 (maintenance) for mutations constructed in *E. coli* MG1655 and *E. coli* EDL933. Growth from low to high numbers in the initiation stage likely depends on utilization of nonlimiting nutrients made available by removing the streptomycin-sensitive facultative anaerobes, whereas persistence during the maintenance stage most likely depends on competition for limiting nutrient(s) (7). Please note that *E. coli* EDL933 does not cause disease in streptomycin-treated CD-1 mice (45).

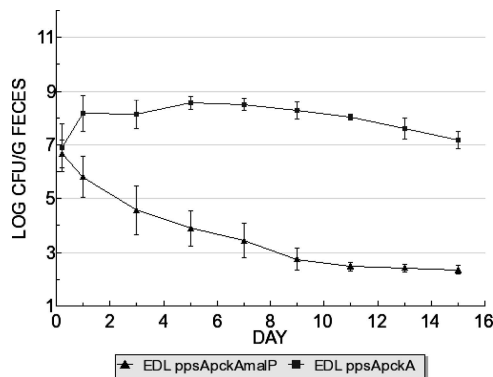


FIG. 1. *E. coli* EDL933 *ppsA pckA malP::mini-Tn5-Km2* STM is outcompeted by *E. coli* EDL933 *ppsA pckA* during colonization. Strains were fed to mice at 10^5 CFU each.

Evidence for in vivo glycogen and maltose metabolism by *E. coli* EDL933. In colonizing the intestine, *E. coli* EDL933 grows from low to high numbers and can do so in the presence of *E. coli* MG1655 (31). To do this, it seems likely that *E. coli* EDL933 uses nutrients that are not used by *E. coli* MG1655, and we recently demonstrated significant differences in monosaccharide utilization between the two strains during colonization of the mouse intestine (16). Previously, we showed that genes involved in glycogen (*glgS*), maltose (*malE*), and maltodextrin (*lamB*) catabolism were significantly induced in *E. coli* MG1655 during growth on mucus, conditions that mimic nutrient availability in the intestine (7). More recently, we found that *E. coli* EDL933 induced *malE* and *glgS* during growth on mucus (16). Lastly, an STM screen identified 14 mutants in an *E. coli* EDL933 *ppsA pckA* background that were unable to colonize streptomycin treated mice in competition with wild-type *E. coli* MG1655. The purpose for using the *ppsA pckA* background was to avoid selection for genes involved in gluconeogenesis (31). One of these insertions was in the *malP* gene. The *E. coli* EDL933 *ppsA pckA malP::mini-Tn5-Km2* STM mutant did not grow on maltose (data not shown), suggesting a polar effect on the downstream *malQ* gene (5). Furthermore, *E. coli* EDL933 *ppsA pckA malP::mini-Tn5-Km2* had a major colonization defect when competed in mice against its *E. coli* EDL933 *ppsA pckA* parent (Fig. 1). Together, these findings led us to consider the importance of maltose and maltodextrin utilization for colonization of the intestine.

Maltose transport is important for intestinal colonization.

The importance of maltose utilization for colonization was determined by testing maltose/maltodextrin ATP binding cassette (ABC) transport system mutants for their ability to compete with the wild-type in streptomycin-treated mice. Null mutations were constructed that deleted the *malEFG* operon encoding the maltose-binding protein and the two membrane-spanning subunits of the maltose ABC transport system, without impacting the adjacent *malK-lamB-malM* operon. *malE*, *malF*, and *malG* mutants are reportedly unable to grow on maltose or maltodextrin (46). Since mutants lacking the maltose ABC transporter can grow on maltose when *malX* is expressed (39), we also made a *malX*-null allele strain, which lacks a maltose phosphotransferase system, as well as a *malEFG malX* double mutant. We verified the phenotypes of

these strains before proceeding with the colonization experiments and found that mutation of *malX* had no effect, whereas mutation of *malEFG* prevented growth on maltose and maltose oxidation (Fig. 2). Mutation of *malEFG* significantly reduced but did not completely eliminate growth on maltodextrin (Fig. 2). We note that growth of a *malEFG* mutant on maltodextrin has not been reported previously (5). Perhaps our *E. coli* EDL933 *malEFG* strain grew poorly on maltodextrin by partially degrading it to glucose in the periplasm by using the MalS periplasmic amylase (19). We did not investigate the maltodextrin phenotype of our *malEFG* mutant further but did confirm the results of the transport mutant colonizations by making independent mutations in the maltose and maltodextrin catabolic pathways, as described below. None of the mutant strains used in the present study affected oxidation of any other carbon sources by comparison to the wild-type on Biolog GN2 plates (data not shown).

E. coli EDL933 *malEFG* was fed together with its wild-type parent, each at 10^5 CFU/mouse. The mutant was able to initiate colonization and reached the same population size as the wild-type on day 1 of the experiment but subsequently declined steadily in numbers, reaching a 3.3-log difference by day 15 (Table 2 and Fig. 3A). *E. coli* EDL933 *malX* cocolonized with the wild-type, indicating that the mutation did not cause a colonization defect (Table 2 and Fig. 3B). The *E. coli* EDL933 *malEFG malX* strain behaved similarly to *E. coli* EDL933 *malEFG* in initiating colonization but subsequently declined in numbers relative to the wild type and reached a 2.3-log difference by the conclusion of the experiment (Table 2 and Fig. 3C). *E. coli* MG1655 differed from *E. coli* EDL933 in having no significant impact of the *malEFG* mutation on colonization (Table 2). Likewise, *E. coli* MG1655 *malX* cocolonized with wild-type *E. coli* MG1655. However, the *E. coli* MG1655 *malEFG malX* double mutant had a colonization defect similar to that of *E. coli* EDL933 *malEFG malX* (Table 2). We conclude from these results that the transport of maltose and/or maltodextrin is important for maintenance of intestinal colonization. To distinguish the involvement of maltodextrin from maltose transport, we knocked out LamB, encoded by *lamB*, a maltodextrin-specific outer membrane porin; *lamB* mutants grow on maltose but cannot grow on maltodextrins (44). This phenotype was verified (Fig. 2). *E. coli lamB* mutants cocolonized with their respective parent strains when fed together to mice (Table 2 and Fig. 3D). From these results, we conclude that the transport of maltose, but not maltodextrins, is important for *E. coli* to colonize the murine intestinal tract.

Maltose catabolism is important for intestinal colonization.

Mutants lacking *malP*, which encodes maltodextrin phosphorylase are unable to catabolize maltodextrin, and *malQ* mutants are unable to catabolize maltose (5). To verify the result of the mini-Tn5 insertion in *malP* (Fig. 1), we constructed *malPQ*-null mutations in the *E. coli* EDL933 and *E. coli* MG1655 backgrounds; both of these *malPQ* mutants were unable to maintain colonization during competition with their respective wild types and had major colonization defects of 3.7 and 3.5 logs, respectively (Table 2 and Fig. 4A). The inability of *E. coli* EDL933 *malPQ* to metabolize either maltose or maltodextrin was confirmed (Fig. 2). It was reported that the growth of *malQ* mutants is inhibited by maltose (5), which could negatively impact competitive fitness in the intestine if maltose was

























Strain	Maltose Growth	Maltose Sensitivity	Maltose Oxidation	Maltodextrin Growth	Maltodextrin Sensitivity	Glycogen
<i>glgA</i>	1.25	1.53		1.24	1.50	
<i>glgP</i>	1.23	1.53		0.69	1.52	
<i>glgS</i>	1.25	1.53		1.24	1.50	
<i>lamB</i>	1.22	1.52		0.07	1.52	
<i>malEFG</i>	0.04	1.50		0.24	1.43	
<i>malX</i>	1.21	1.53		1.23	1.51	
<i>malEFG malX</i>	0.03	1.47		0.25	1.47	
<i>malP</i>	1.25	1.52		0.03	1.52	
<i>malQ</i>	0.03	1.47		1.22	1.52	
<i>malPQ</i>	0.04	1.51		0.03	1.51	
<i>malP glgP</i>	1.24	1.52		0.04	1.52	
WT	1.25	1.53		1.25	1.53	

FIG. 2. Phenotypes of *E. coli* EDL933 mutants used in the present study. Growth on maltose or maltodextrin as the sole carbon source was determined in MOPS medium containing 0.2% carbohydrate. Maltose and maltodextrin sensitivity was determined in LB containing 0.2% carbohydrate. The final OD₆₀₀ values of cultures at 24 h are shown. Oxidation of maltose was determined on Biolog GN2 phenotype plates, as shown in graphlets of relative amounts of substrate oxidized (y axis) versus time. Glycogen accumulation on LB agar containing 2% glucose was visualized by staining with iodine vapor.

present. For this reason, we tested the mutant strains used here for maltose and maltodextrin sensitivity; none of the strains tested were sensitive to maltose or maltodextrin (Fig. 2). Since the *E. coli* EDL933 *malPQ* population in the competitive colonization assay was only 1 log above the limit of detection (10³ CFU/g of feces), we wanted to test whether the observed colonization defect resulted from an inability to grow in the intestine or an inability to compete with the wild type; the latter was confirmed, since both *malPQ* mutant strains colonized at wild-type levels when fed alone to mice (data not shown). Thus, we conclude that the inability of the *E. coli malPQ* mutants to catabolize maltose and/or maltodextrin strongly and negatively impacted competitive fitness in the mouse intestine, which explains why the STM experiment revealed a *malP* insertion mutant.

To distinguish the catabolism of maltose from that of maltodextrin, individual null mutations were constructed in *malP* and *malQ*, which eliminated growth on maltodextrin and maltose, respectively (5). Before proceeding with the colonizations, we verified these phenotypes (Fig. 2). *E. coli* EDL933 *malQ* was fed together with wild-type *E. coli* EDL933 and observed to have a 3.3-log colonization defect (Table 2 and Fig. 4B), confirming that maltose catabolism is important for coloniza-

tion. The *malP* mutation had a modest, but statistically insignificant, impact on colonization of *E. coli* EDL933 (Table 2 and Fig. 4C). This result, together with the lack of a colonization defect for the *lamB* mutant (Fig. 3C), suggests that maltodextrins are not important substrates in the intestine. These results confirm the results obtained with the transporter mutants, i.e., that the ability to use maltose, but not maltodextrins, is important for intestinal colonization.

Maltose is necessary for *E. coli* EDL933 to initiate colonization during competition with *E. coli* MG1655. To test whether maltose metabolism is important for the pathogen *E. coli* EDL933 to compete with the commensal *E. coli* MG1655 in the mouse intestine, 10⁵ CFU of the *E. coli* EDL933 *malQ* mutant was fed to mice together with 10¹⁰ CFU of wild-type *E. coli* MG1655. The results show that *E. coli* EDL933 *malQ* was unable to initiate in competition with wild-type *E. coli* MG1655 during colonization, whereas the wild-type *E. coli* EDL933 initially outcompetes *E. coli* MG1655 (compare Fig. 5A and Fig. 5B). This result indicates that maltose is used by pathogenic *E. coli* EDL933 to grow from low to high numbers while attempting to gain residence in the mouse intestine when higher numbers of commensal *E. coli* MG1655 are present.

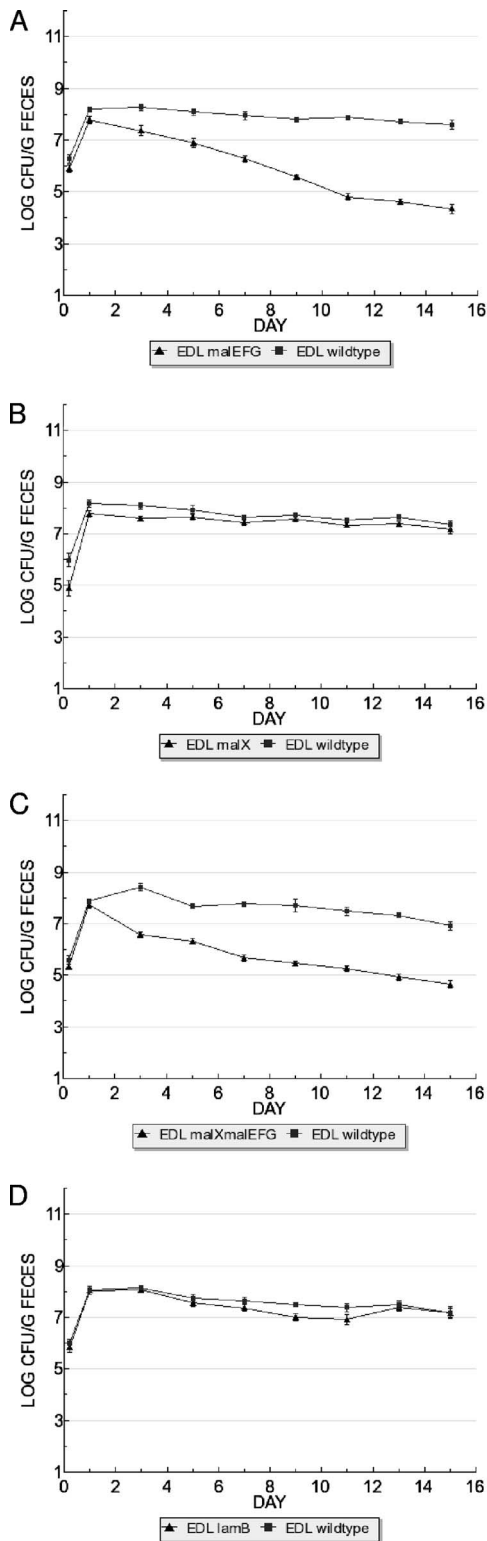


FIG. 3. Maltose/maltodextrin transport mutants display colonization defects. (A) *E. coli* EDL933 $\Delta(malE-malG)$ has a colonization defect in competition with *E. coli* EDL933 wild-type. (B) *E. coli* EDL933 $\Delta malX$ does not exhibit a colonization defect. (C) *E. coli* EDL933 $\Delta malX \Delta(malE-malG)$ has a colonization defect in competition with *E. coli* EDL933 wild-type. (D) *E. coli* EDL933 $\Delta lamB$ does not exhibit a colonization defect. Strains were fed to mice at 10^5 CFU each.

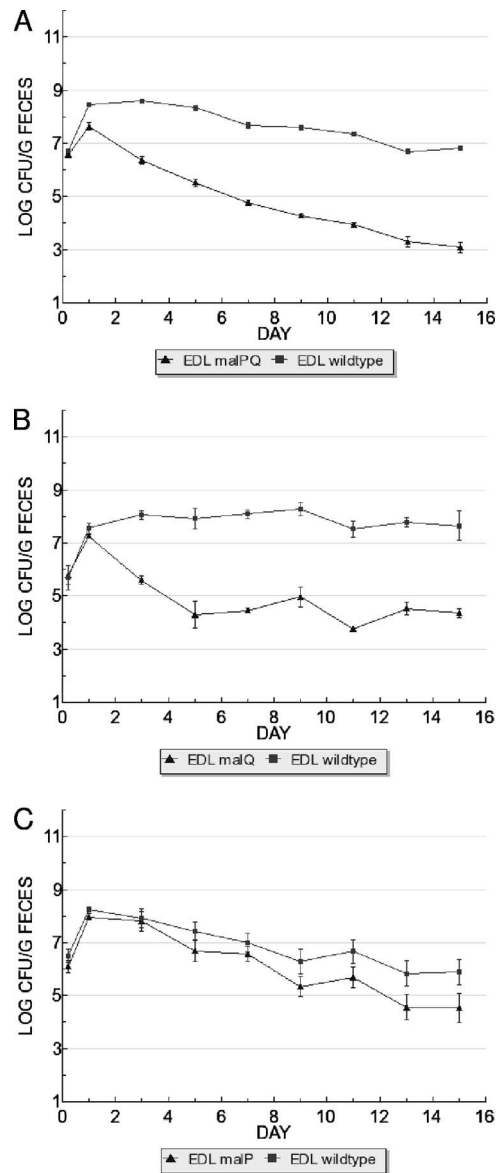


FIG. 4. Catabolism of maltose but not maltodextrins is important for competitive colonization of the mouse intestine. (A) *E. coli* EDL933 $\Delta(malP-malQ)$ has a colonization defect in competition with *E. coli* EDL933 wild-type. (B) *E. coli* EDL933 $\Delta malQ$ has a colonization defect in competition with *E. coli* EDL933 wild-type. (C) *E. coli* EDL933 $\Delta malP$ does not exhibit a significant colonization defect. Strains were fed to mice at 10^5 CFU each.

Glycogen synthesis is important for colonization of the intestine. To test whether the ability to make glycogen (i.e., for carbon storage) provides an advantage in the intestine, null mutations were constructed in two of the genes that are necessary for glycogen synthesis (*glgA* and *glgS*), the phenotypes of which were previously described (21, 26). Mutation of *glgA*, which encodes glycogen synthetase (26), resulted in a glycogen-negative phenotype; mutation of *glgS*, which encodes a factor that plays an important and yet unknown role in glycogen synthesis (21), resulted in a reduced amount of glycogen; neither mutation affected metabolism of maltose or maltodex-

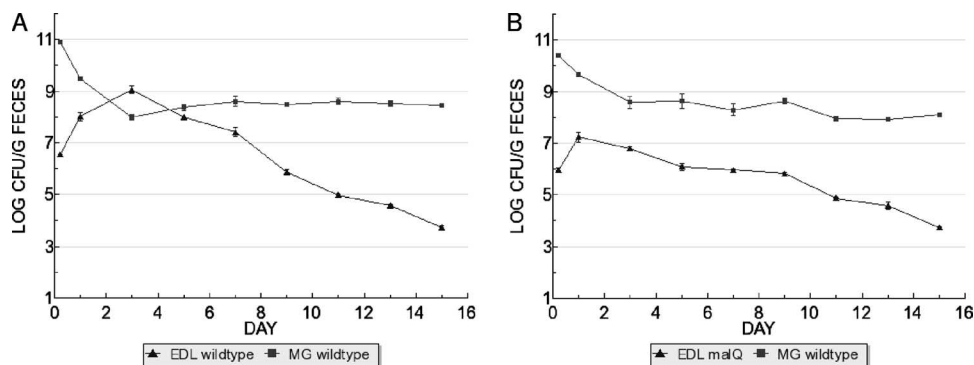


FIG. 5. Catabolism of maltose is necessary for *E. coli* EDL933 to compete efficiently with wild-type *E. coli* MG1655 in the mouse intestine. (A) *E. coli* EDL933 wild-type (fed to mice at 10^5 CFU) initially outcompetes higher numbers of *E. coli* MG1655 wild-type (fed at 10^{10} CFU). (B) *E. coli* EDL933 $\Delta malQ$ (fed at 10^5 CFU) cannot compete with higher numbers of *E. coli* MG1655 wild-type (fed at 10^{10} CFU).

trin (Fig. 2). *E. coli* EDL933 and *E. coli* MG1655 *glgA* mutants had colonization defects of 2.3 and 2.9 logs, respectively, in competition with their wild-type parents (Table 2 and Fig. 6). Likewise, *E. coli* EDL933 and *E. coli* MG1655 *glgS* mutants had colonization defects of 1.9 and 1.6 logs, respectively, in competition with their wild-type parents (Table 2 and Fig. 6). Thus, we conclude that that ability to synthesize carbon stores in the form of glycogen is important for *E. coli* to colonize the mouse intestine.

Glycogen degradation is important for colonization of the intestine. We wanted to test whether the ability to degrade glycogen provides a competitive advantage in the mouse intestine. The primary pathway for glycogen degradation involves glycogen phosphorylase, which is encoded by *glgP*. Although the pathways for metabolism of glycogen and maltodextrin are similar, they are not inextricable, i.e., although they have similar α -glucan phosphorylase activities, GlgP cannot replace MalP in *E. coli* (5). Mutants were constructed in *glgP*, which are unable to breakdown glycogen but could still catabolize maltose and maltodextrin, as described previously (1). Before proceeding with the colonization, we tested the phenotype and found the *E. coli* EDL933 *glgP* strain was glycogen positive, showed modestly reduced growth on maltodextrin compared to the wild type, and metabolized maltose normally (Fig. 2). *E. coli* EDL933 and *E. coli* MG1655 *glgP* mutants could initiate

but not maintain colonization, as indicated by 2.4- and 3.2-log colonization defects, respectively, by day 15 postfeeding (Table 2 and Fig. 7). Apparently, MalP did not contribute to glycogen degradation in vivo, since the *malP* mutation alone did not significantly affect colonization and the *glgP malP* double mutants had colonization defects of 2.5 logs at day 15, which were similar to the colonization defects of the *glgP* mutants (Table 2). These results provide additional evidence that glycogen is an important carbon store that provides a colonization advantage in the intestine.

Evidence for a hunger or famine existence in the intestine. Since glycogen is known to promote the survival of *E. coli* (42), our finding that glycogen synthesis and degradation is necessary for efficient colonization suggests that *E. coli* encounters times of carbon limitation in the intestine that can be offset by glycogen carbon stores. To confirm this possibility, we used a strategy designed to provide the microbiota with a steady, high level of a rapidly metabolized carbon source. We previously showed that gluconate, which supports the growth of *E. coli* but is not absorbed by the host, is able to affect the outcome of experiments that depend on competition for gluconate (28). We reasoned that the constant availability of an excess supply of gluconate, provided by adding 2% gluconate to the drinking water of mice throughout the experiment, would rescue the dependence of colonized *E. coli* on stored glycogen. Indeed,

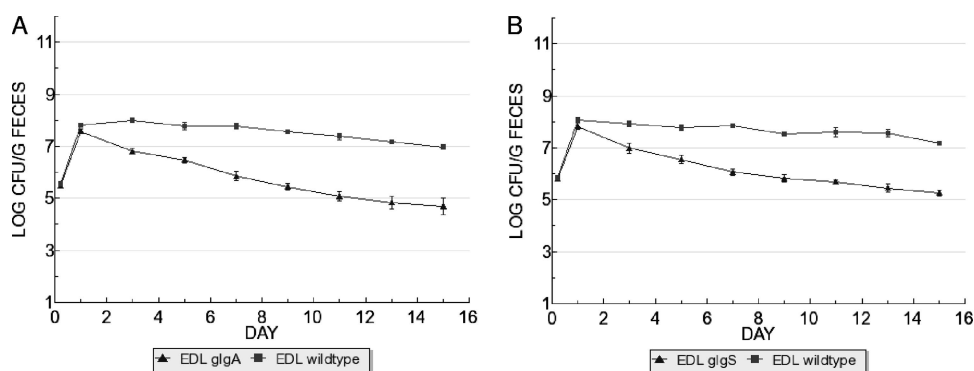


FIG. 6. Glycogen synthesis mutants do not compete effectively in the mouse intestine. (A) *E. coli* EDL933 $\Delta glgA$ exhibits colonization defect in competition with *E. coli* EDL933 wild-type. (B) *E. coli* EDL933 $\Delta glgS$ exhibits colonization defect in competition with *E. coli* EDL933 wild-type. Strains were fed to mice at 10^5 CFU each.

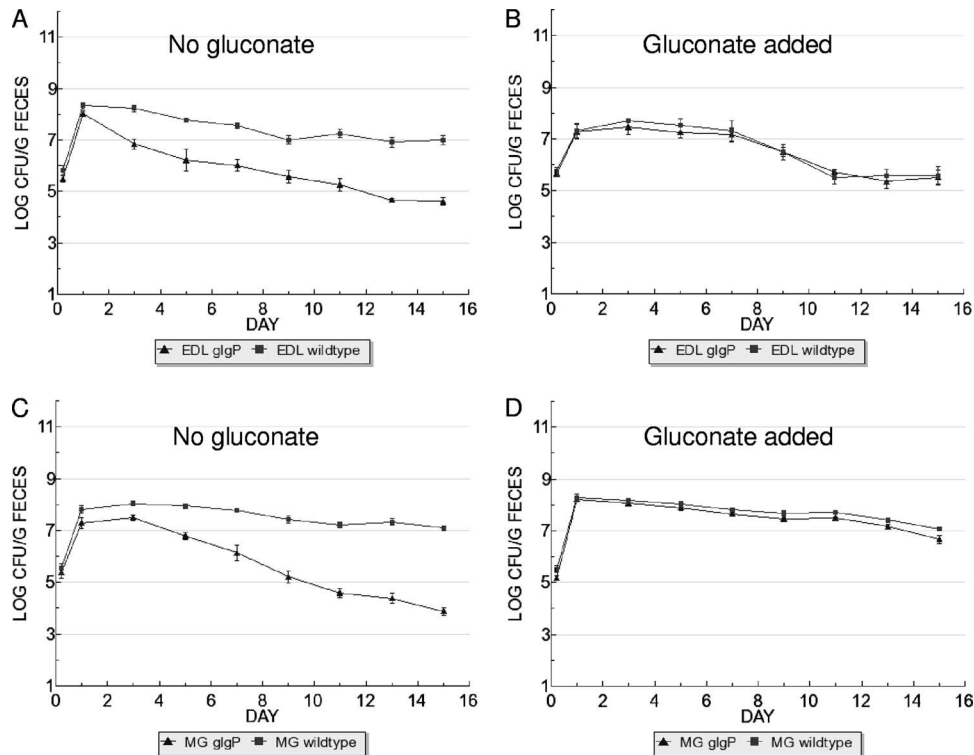


FIG. 7. Colonization defects of glycogen degradation mutants are rescued by adding 2% gluconate to drinking water of mice. (A) *E. coli* EDL933 Δ glgP and (C) *E. coli* MG1655 Δ glgP exhibit colonization defects in competition with their respective wild-type parents. (B and D) These colonization defects are rescued by addition of gluconate to the drinking water. Strains were fed to mice at 10^5 CFU each.

the *E. coli* EDL933 and *E. coli* MG1655 *glgP* mutants were equally fit for colonization in competition with their wild-type parents when gluconate was supplied in the drinking water (Fig. 7). Likewise, the addition of 2% gluconate to the drinking water rescued the colonization defect of *E. coli* EDL933 *glgA* (data not shown). These results support the conclusion that glycogen is important for maintaining the highest possible energy levels during times of carbon limitation in the intestine.

DISCUSSION

In this study, we examined the metabolism of maltose, maltodextrin, and glycogen by pathogenic and commensal *E. coli* in the mouse intestine. The results show that catabolism of the disaccharide maltose provides a competitive advantage in vivo, whereas degradation of exogenous forms of the more complex glucose polymer, maltodextrin, does not. Furthermore, we found that the utilization of maltose in vivo gives the pathogen *E. coli* EDL933 a competitive advantage during the initiation stage of colonization when competing with commensal *E. coli* MG1655. The endogenous glucose polymer, glycogen, appears to play a very important role in colonization. In support of the hypothesis that *E. coli* relies on internal carbon stores to maintain colonization during periods of famine, we found that by providing a constant supply of a readily metabolized sugar, i.e., gluconate, in the drinking water, the competitive disadvantage of *E. coli* glycogen metabolism mutants (e.g., *glgP*) was rescued.

The hypothesis that glycogen provides a source of internal

carbon and energy storage and thus is advantageous for animal colonization and virulence was considered previously. However, the results from the two published studies are in conflict and neither provided a direct test of the hypothesis. Bonafonte et al. (4) showed a correlation between the amount of glycogen stores in *Salmonella enterica* serovar Enteritidis and the 50% lethal dose in 1-day-old chicks. In that study, there was no way to know whether the stored glycogen was used by the bacteria during the course of infection. In another study, McMeehan et al. (30) focused on the role of ADP-glucose pyrophosphorylase, encoded by *glgC*, in *Salmonella enterica* serovar Typhimurium. These authors found no significant differences in intestinal colonization or virulence (in 1-day-old chicks) between strains carrying *glgC* mutations or wild-type alleles in three different serovars (Gallinarum, Pullorum, or Typhimurium) (30). The *glgC* strains were thought not to accumulate glycogen because they cannot make the glycogen precursor, ADP-glucose. However, that conclusion is brought into question by the recent finding that *glgC* mutants of *E. coli* and *S. enterica* serovar Typhimurium possess an alternative pathway for ADP glucose formation and can accumulate glycogen under certain conditions (33).

The experiments described here directly tested the hypothesis that synthesis and utilization of glycogen is necessary for efficient colonization of the intestine by *E. coli*. Mutants lacking *GlgA*, which cannot synthesize glycogen, had significant colonization defects in competition with the wild-type strain that accumulates glycogen normally. Likewise, mutants lacking *GlgP*, which cannot degrade glycogen, had similarly significant

colonization defects. Loss of GlgS, which is known to impair glycogen metabolism, resulted in a colonization defect similar to those of mutants lacking GlgA or GlgP. These data provide strong evidence in support of the hypothesis that glycogen metabolism is important in colonized *E. coli*. The need for glycogen stores may reflect a physiological status of nutrient limitation in the intestine. Accordingly, glycogen stores might be consumed by colonized bacteria as they cometabolize mixtures of severely limiting carbon sources or switch from one substrate to another. Alternatively, carbon availability in the intestine might be intermittent, requiring the cell to obtain maintenance energy from glycogen during brief periods of famine. If it is true that glycogen provides a source of carbon and energy during times of nutrient limitation, we reasoned that by providing a constant source of a readily metabolized carbon source the colonization defect of a *glgP* mutant would be rescued. We previously established that gluconate, which is not absorbed in the mammalian gastrointestinal tract, is available to strains that can use it and can impact colonization, strongly suggesting that gluconate provided in the drinking water of mice is metabolized by *E. coli* in the intestine (28). Indeed, we found that addition of gluconate to the drinking water made *glgP* mutants better colonizers, equal to wild-type *E. coli* (Fig. 7). These results indicate that glycogen stores not only support the long-term survival of *E. coli* outside of the host (42) but also enhance persistence in the intestine.

The biochemistry of glycogen metabolism is ideally suited to provide energy during brief respites from exogenous carbon and energy sources. The genes for glycogen synthesis and degradation are arranged together in an operon and are coregulated, such that the enzymes of both glycogen synthesis and degradation are coexpressed (37). At first, this may seem paradoxical but, in fact, since both sets of enzymes are present, the cell is poised at any given moment to either make glycogen or degrade it, as conditions allow. Recall that glycogen is formed when carbon is in excess but some other nutrient is limiting and glycogen is degraded when carbon is limiting (13, 36). Moreover, glycogen is synthesized from glucose-1-phosphate, which is also the breakdown product of glycogen. Since the enzymes of glycogen synthesis and degradation are coexpressed, their activities (i.e., accumulation or catabolism of glycogen) are reciprocally controlled by the physiological constraints of carbon source excess and depletion, both of which are reflected in the intracellular pool of glucose-1-phosphate, which in turn reflects the energy status of the cell (5). If nutrient limitation prevails in the intestine and if this limitation includes, periodically, nutrients other than carbon sources (i.e., nitrogen), then the biochemical regulation of glycogen metabolism is ideal for responding to the instantaneous excesses and limitations of carbon sources and other nutrients. Perhaps the reason that glycogen storage is widespread in enteric bacteria is because this is a necessary strategy for rapid growth in the intestine, where there is intense competition for resources.

While *E. coli* apparently depends on endogenous stores of the polysaccharide, glycogen, it cannot metabolize exogenous polysaccharides, with the exception of maltodextrin (29). The data presented here indicate that maltodextrin does not support colonization. Thus, the in vivo catabolic capacity of *E. coli* must be limited primarily to mono- and disaccharides. Previous in vivo studies of *E. coli* catabolism focused on monosaccha-

rides (7). We have, however, now studied a few disaccharides and found that lactose does not support colonization by *E. coli* EDL933 and *E. coli* MG1655 (16), nor do β -glucuronides support colonization by *E. coli* MG1655 (7). The in vivo catabolism of α -glycosides, i.e., trehalose and sucrose, and α -galactosides, i.e., melibiose, has not been studied and should be, but at least one disaccharide, maltose, supports colonization by *E. coli* EDL933 and *E. coli* MG1655.

Clearly, growth on intestinal polysaccharides is the domain of the anaerobes that secrete polysaccharide hydrolyzing enzymes (10, 15, 41). Some, if not all, dominant intestinal anaerobes preferentially utilize oligosaccharides over mono- and disaccharides (2). Moreover, at least some minor members of the intestinal microbiota grow by cross-feeding on sugars released by the polysaccharide degraders (17, 40). For example, there is recent evidence that vancomycin-resistant *Enterococcus* colonizes the intestine by growing on monosaccharides released by breakdown of complex polysaccharides by the indigenous microbiota (38). As we suggested previously (7) and in accordance with the findings summarized here, *E. coli* almost certainly colonizes the intestine by cross-feeding on simple sugars provided by anaerobic polysaccharide degradation. These sugars must be of limited availability, since *E. coli* relies on endogenous carbon stores to efficiently colonize the intestine. Therefore, changes in the microbiota and/or polysaccharide availability that further limit the availability of the nutrients preferred by *E. coli* would limit its colonization. Importantly, we take this to mean that there is merit to combating enteric infections by using probiotics or prebiotics (20) to manipulate the intestinal microbiota in such a way as to limit the availability of sugars preferred by *E. coli* O157:H7 (16) and perhaps other pathogens. Accordingly, prebiotics would have to stimulate populations that consume the sugars favored by invading pathogens, while probiotics could accomplish this directly, since organisms could be engineered to specifically consume preferred nutrients.

Studies of the physiology of carbohydrate metabolism and its contribution to pathogenesis are yielding novel, broadly applicable insights into host-pathogen interactions, which are specifically related to the underlying mechanisms that enable pathogens to successfully colonize their hosts. Many of the nutrient acquisition systems used by *E. coli* are similar to those used by other mucosal pathogens. Therefore, such studies will aid in finding new and effective treatments. In conclusion, we wish to emphasize that therapies designed to specifically reduce intestinal nutrient availability may be effective because organisms such as *E. coli* O157:H7 live a hunger or famine existence, as indicated by their requirement for glycogen stores, which places the pathogens in peril of starvation before infection can begin, if administration of prebiotics or probiotics depletes their preferred nutrients.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant AI48945 to T.C. and P.S.C. Partial support for C.S. was provided by Danish Research Agency Grant 2052-03-0013.

We thank April B. Anderson for the construction of some strains used in this study and Susanne Schjøring for assisting with colonization experiments.

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