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Escherichia coli EDL933 Requires Gluconeogenic Nutrients To Successfully Colonize the Intestines of Streptomycin-Treated Mice Precolonized with E. coli Nissle 1917

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Escherichia coli MG1655, a K-12 strain, uses glycolytic nutrients exclusively to colonize the intestines of streptomycin-treated mice when it is the only E. coli strain present or when it is confronted with E. coli EDL933, an O157:H7 strain. In contrast, E. coli EDL933 uses glycolytic nutrients exclusively when it is the only E. coli strain in the intestine but switches in part to gluconeogenic nutrients when it colonizes mice precolonized with E. coli MG1655 (R. L. Miranda et al., Infect Immun 72:1666–1676, 2004, http://dx.doi.org/10.1128/IAI.72.3.1666-1676.2004). Recently, J. W. Njoroge et al. (mBio 3:e00280-12, 2012, http://dx.doi.org/10.1128/mBio.00280-12) reported that E. coli 86-24, an O157:H7 strain, activates the expression of virulence genes under gluconeogenic conditions, suggesting that colonization of the intestine with a probiotic E. coli strain that outcompetes O157:H7 strains for gluconeogenic nutrients could render them nonpathogenic. Here we report that E. coli Nissle 1917, a probiotic strain, uses both glycolytic and gluconeogenic nutrients to colonize the mouse intestine between 1 and 5 days postfeeding, appears to stop using gluconeogenic nutrients thereafter in a large, long-term colonization niche, but continues to use them in a smaller niche to compete with invading E. coli EDL933. Evidence is also presented suggesting that invading E. coli EDL933 uses both glycolytic and gluconeogenic nutrients and needs the ability to perform gluconeogenesis in order to colonize mice precolonized with E. coli Nissle 1917. The data presented here therefore rule out the possibility that E. coli Nissle 1917 can starve the O157:H7 E. coli strain EDL933 of gluconeogenic nutrients, even though E. coli Nissle 1917 uses such nutrients to compete with E. coli EDL933 in the mouse intestine.

While much attention has been paid to the role of specific virulence factors in bacterial pathogenesis, until recently little attention has been paid to the roles of specific metabolic pathways in the ability of bacterial pathogens to initiate the pathogenic process. Yet clearly, if a bacterial pathogen is unable to initiate pathogenesis because nutrients essential for its growth are unavailable in the host, it will be unable to establish itself and cause disease. Therefore, it is important to determine whether pathogens require glycolytic nutrients, gluconeogenic nutrients, or both for success in invading and subsequently colonizing the host. Indeed, recent studies have suggested that some pathogens use glycolytic nutrients, some use gluconeogenic nutrients, and some use both to adapt to diverse host habitats (1–7).

We are interested in the nutritional basis of Escherichia coli colonization of the intestine and particularly in whether precolonization by commensal E. coli strains can be used to prevent invading E. coli intestinal pathogens from colonizing. Commensal E. coli strains colonize the human intestine in the presence of a dense and diverse intestinal microbiota comprising at least 500 cultivable species and 10^{13} to 10^{14} total bacteria (8). Unfortunately, E. coli colonization cannot be studied experimentally in conventional animals due to colonization resistance, which occurs when all niches are filled by the microbiota (9). Such experiments require an animal model with open niches for E. coli to colonize the intestine in relatively high numbers, but to be authentic, the model intestine must also have a dense and diverse anaerobic community that matches the native microbiota of the conventional animal as closely as possible. The streptomycin-treated mouse model is used routinely for this purpose, since streptomycin-treated mice fulfill these criteria (10, 11). Using this model, we have shown that for E. coli to colonize, it must obtain nutrients in the mucus layer, which overlies the epithelium (10, 11), that it resides in the mucus layer as a member of mixed biofilms (12, 13), and that each E. coli strain displays a unique nutritional program in the intestine (14).

E. coli strains can utilize both glycolytic and gluconeogenic nutrients for growth in vitro, and we reported previously that both E. coli MG1655, a commensal strain, and E. coli EDL933, an O157:H7 pathogenic strain, use glycolytic but not gluconeogenic nutrients to colonize the intestines of streptomycin-treated mice when each is the only E. coli strain fed to mice (15). However, we also reported that in the presence of E. coli MG1655, E. coli EDL933 switches, at least in part, to gluconeogenesis in order to maintain itself in the intestine, while E. coli MG1655 continues to use glycolytic nutrients exclusively (15). These findings are of extreme interest in view of recent reports showing that E. coli 86-24, also an O157:H7 strain, activates the expression of virulence genes...
under gluconeogenic conditions (16, 17), because this suggests the possibility that precolonization of the intestine with a probiotic E. coli strain that starves E. coli O157:H7 strains of gluconeogenic nutrients might render them nonpathogenic.

E. coli Nissle 1917 is a commensal strain that has been used as a probiotic agent to treat gastrointestinal infections in humans since the early 1920s (18). Several features of E. coli Nissle 1917 have been proposed to be responsible for its probiotic nature, including its ability to express two microcins (19), the absence of known protein toxins, its semirough lipopolysaccharide and hence its serum sensitivity (20, 21), and the presence of six iron uptake systems (22). More recently, it has been shown that E. coli Nissle 1917 inhibits the adherence of E. coli EDL933 to human epithelial cells in vitro and simultaneously inhibits its growth and Shiga toxin 2 (Stx2) production (23). It has also been shown that in the intestines of streptomycin-treated mice, E. coli Nissle 1917 is better than a number of other commensal E. coli strains at limiting the growth of E. coli EDL933 (12, 13). In this study, we examine whether E. coli Nissle 1917 uses both glycolytic and gluconeogenic nutrients to colonize the intestines of streptomycin-treated mice and whether E. coli EDL933 switches to gluconeogenic nutrients in order to maintain itself in the presence of E. coli Nissle 1917.

**MATERIALS AND METHODS**

**Bacterial strains.** The bacterial strains used in this study are listed in Table 1. The original E. coli K-12 strain was obtained from a stool sample from a convalescing diphtheria patient in Palo Alto, CA, in 1922 (24). The sequenced E. coli MG1655 strain (CGSC 7740) was derived from the original K-12 strain, having only been cured of the temperate bacteriophage lambda and the F plasmid by means of UV light and acridine orange treatment (24). It has an IS1 element in the fhlDC promoter (25). E. coli EDL933 is the prototype strain of enterohemorrhagic E. coli (EHEC) O157:H7 (26). E. coli Nissle 1917 was originally isolated during World War I from a soldier who escaped a severe outbreak of diarrhea (18). It has a beneficial effect on several types of intestinal disorders, is well tolerated by humans, and has been marketed as a probiotic remedy against intestinal disorders in several European countries since the early 1920s (18).

**Media and growth conditions.** LB broth (Lennox) (Difco Laboratories, Detroit, MI), LB agar (Lennox) (Difco), and MacConkey agar (Difco) were used for routine cultivation. SOC medium was prepared as described by Datsenko and Wanner (27). For testing carbon and energy source utilization in the Biolog system, M9 minimal medium (28) was modified by the addition of 120 mM NaCl to more closely approximate the sodium chloride concentration in the intestine (29).

### Table 1: Bacterial strains

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Genotype or phenotype</th>
<th>Designation in the text</th>
<th>Source or reference</th>
</tr>
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<tbody>
<tr>
<td>Nissle 1917 St†</td>
<td>Spontaneous streptomycin-resistant mutant of E. coli Nissle 1917</td>
<td>E. coli Nissle 1917</td>
<td>42</td>
</tr>
<tr>
<td>Nissle 1917 St† Nal †</td>
<td>Spontaneous nalidixic acid-resistant mutant of E. coli Nissle 1917 St†</td>
<td>E. coli Nissle 1917</td>
<td>42</td>
</tr>
<tr>
<td>Nissle 1917 St† ppsA ΔpckA ΔppsA ΔpckA::cat</td>
<td>chloramphenicol resistance cassette in the ppsA deletion; unable to metabolize gluconeogenic substrates</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>MG1655 Str† Nal †</td>
<td>Spontaneous streptomycin- and nalidixic acid-resistant mutant of E. coli MG1655</td>
<td>E. coli MG1655</td>
<td>15</td>
</tr>
<tr>
<td>EDL933 St†</td>
<td>Spontaneous streptomycin-resistant mutant of E. coli EDL933</td>
<td>E. coli EDL933</td>
<td>15</td>
</tr>
<tr>
<td>EDL933 St† Rif †</td>
<td>Spontaneous rifampin-resistant mutant of E. coli EDL933 St†</td>
<td>E. coli EDL933</td>
<td>36</td>
</tr>
<tr>
<td>EDL933 St† ΔppsA ΔpckA ΔpckA::cat</td>
<td>chloramphenicol resistance cassette in the ppsA deletion; unable to metabolize gluconeogenic substrates</td>
<td>E. coli EDL933</td>
<td>15</td>
</tr>
</tbody>
</table>

**Mutant construction.** The allelic exchange method described by Datsenko and Wanner (27) was used to construct E. coli Nissle 1917 St† ΔpckA ΔppsA ΔpckA::cat (Table 1). The method allows the sequential construction of double deletion mutants by use of a removable chloramphenicol cassette (27). The primers used to construct the deletion mutants were designed according to the genome sequence of E. coli CFT073 (30), which is closely related to that of E. coli Nissle 1917 (22). Deletions of 1,327 bp and 2,367 bp were made in the pckA gene and the ppsA gene, respectively. The pckA deletion primers used to make E. coli Nissle 1917 St† ΔpckA ΔpckA::cat were as follows: primer 1, 5′-AGTCTTTACTATCGACATACATAT TGGCTAAGGAGCAGTGgtgtaggctggagctgcttcg-3′; primer 2, 5′-GGCTAAGGAGCAGTGgtgtaggctggagctgcttcg-3′; primer 3, 5′-GGCTAAGGAGCAGTGgtgtaggctggagctgcttcg-3′; primer 4, 5′-GGCTAAGGAGCAGTGgtgtaggctggagctgcttcg-3′. DNA sequencing was carried out at the URI Genomics and Sequencing Center, University of Rhode Island, Kingston, RI, using an Applied Biosystems 3130xl genetic analyzer (Applied Biosystems, Foster City, CA). A BigDye Terminator cycle sequencing kit (version 3.1; Applied Biosystems) was used for the sequencing reactions.

**Biolog phenotypic analysis.** For confirmation that E. coli Nissle 1917 St† ΔpckA ΔppsA ΔpckA::cat was able to grow on glycolytic substrates but unable to grow on gluconeogenic substrates, this strain and its wild-type parent were grown overnight in M9 minimal medium containing 0.4% (vol/vol) glycerol and 0.2 M NaCl at 37°C. Cultures were washed twice in M9 minimal medium containing 0.2 M NaCl without any carbon source and then diluted to an optical density at 600 nm (OD600) of 0.06 in 5 ml of M9 minimal medium without any carbon source. Each culture was diluted an additional 10-fold, and 100 μl was added to a 96-well microtiter plate (Biolog GN2 MicroPlate). Plates were incubated at 37°C for 48 h in the OmniLog system. Levels of reduction of tetrazolium violet dye were measured at 15-min intervals and were plotted against time, resulting in a kinetic curve. E. coli Nissle 1917 and E. coli Nissle 1917 ΔpckA ΔppsA ΔpckA::cat each displayed the highest final level of tetrazolium reduction when either glucose or glycerol was used as the sole carbon source. Final levels of tetrazolium reduction on all other carbon sources were normalized to that on glycerol.
Mouse colonization experiments. The specifics of the streptomycin-treated mouse model, used to compare the large-intestine-colonizing abilities of E. coli strains in mice, have been described previously (12). Briefly, sets of three male CD-1 mice (5 to 8 weeks old) were given drinking water containing streptomycin sulfate (5 g/liter) for 24 h to eliminate resident facultative bacteria (31). Following 18 h of starvation for food and water, the mice were fed 1 ml of 20% (wt/vol) sucrose containing 10^5 CFU of LB broth (Lennox)-grown E. coli strains, as described in Results. After ingestion of the bacterial suspension, both the food (Teklad mouse and rat diet; Harlan Laboratories, Madison, WI) and the streptomycin-containing water were returned to the mice, and 1 g of feces was collected after 5 h and 24 h and on odd-numbered days, at the times indicated in the figures. Mice were housed individually in cages without bedding and were placed in clean cages at 24-h intervals. Individual fecal pellets were therefore no older than 24 h. Each fecal sample (1 g) was homogenized in 10 ml of 1% Bacto tryptone (Difco), diluted in the same medium, and plated on MacConkey agar plates with appropriate antibiotics. When appropriate, 1 ml of a fecal homogenate (sampled after the large debris had settled) was centrifuged at 12,000 × g, resuspended in 100 μl of 1% Bacto tryptone, and plated on a MacConkey agar plate with appropriate antibiotics. This procedure increased the sensitivity of the assay from 10^2 CFU/g of feces to 10 CFU/g of feces. To distinguish the various E. coli strains in feces, dilutions were plated on lactose MacConkey agar containing streptomycin sulfate (100 μg/ml) either alone or together with nalidixic acid (50 μg/ml), chloramphenicol (30 μg/ml), or rifampin (50 μg/ml). Streptomycin sulfate, chloramphenicol, and nalidixic acid were purchased from Sigma-Aldrich (St. Louis, MO). All plates were incubated for 18 to 24 h at 37°C prior to counting. Each colonization experiment was performed at least twice, with essentially identical results. Pooled data from at least two independent experiments (for a total of six mice) are presented in the figures. All animal protocols were approved by the University Committee on Use and Care of Animals at the University of Rhode Island.

FISH. Fluorescence in situ hybridization (FISH) was performed as described previously (12, 32). Briefly, the cecum of a mouse designated for FISH was cut into 1- to 2-cm pieces, suspended in Tissue-Tek OCT compound (Sakura, Torrance, CA), and snap-frozen in 2-methylbutane suspended in liquid nitrogen. Pieces were then stored at −80°C until ready for sectioning. For sectioning, the UltraPro 5000 cryostat (Vibratome, St. Louis, MO) was used to cut 10-μm sections, which were adhered to poly-l-lysine-treated slides for visualization. Slides were fixed in 4% paraformaldehyde for 1 h at room temperature, followed by a wash in phosphate-buffered saline for 10 min. Slides were then allowed to air dry overnight prior to hybridization. Fluorescent probes (Integrated DNA Technologies [IDT], Coralville, IA) were diluted in hybridization solution (0.1 M Tris-HCl buffer [pH 7.2], 0.9 M NaCl, and 0.1% SDS). The E. coli 23S rRNA-specific Cy3-conjugated probe (5’–Cy3–CAC CGT AGT GCC TCG TCA TCA–3’) and the ebubacterial 23S rRNA-specific FAM–(6-carboxyfluorescein) probe (5’–GCT GCC TCC CGT AGG FAM–3’) were used at concentrations of 5 ng/μl and 25 ng/μl, respectively. Subsequently, 10 μl of diluted probes in hybridization solution was pipetted onto each slide. Each slide was then covered with a HybriSlip hybridization cover (Life Technologies, Carlsbad, CA) and was allowed to incubate in the dark for 2 h at 50°C. The slides were then placed in a wash buffer (0.1 M Tris-HCl buffer [pH 7.2] and 0.9 M NaCl) and were allowed to soak for 20 min at 50°C. Slides were then rinsed with distilled water and were air dried overnight. Sections were viewed by confocal microscopy. Prior to viewing, sections on the poly-l-lysine treated slides were treated with Vectashield mounting medium (Vector Laboratories, Burlingame, CA), an anti-bleaching agent that helps prolong fluorescence for imaging.

Statistics. The colonization data for E. coli EDL933 from day 17 through day 21 in Fig. 3A were combined, as were the corresponding data in Fig. 6A, and these Fig. 3A data and Fig. 6A data were compared by a two-tailed Student t test. Also, the colonization data for E. coli MG1655 from day 17 through day 21 in Fig. 2A and B were combined, as were those for E. coli Nissle 1917 from day 17 through day 21 in Fig. 3A and B, and these data for the two strains were compared by a two-tailed Student t test. P values of <0.05 were interpreted as indicating a significant difference.

RESULTS

E. coli Nissle 1917 uses glycolytic and gluconeogenic nutrients for growth in the mouse intestine. We reported previously that both E. coli MG1655, a commensal strain, and E. coli EDL933, an O157:H7 strain, use glycolytic but not gluconeogenic nutrients to colonize the intestines of streptomycin-treated mice when each is the only E. coli strain fed to mice (15). It was of interest to determine whether the same is true of E. coli Nissle 1917, an effective probiotic strain (18). Phosphoenolpyruvate carboxykinase converts oxaloacetate to phosphoenolpyruvate and is encoded by the E. coli pckA gene (33). Phosphoenolpyruvate synthase is encoded by the E. coli ppsA gene and converts pyruvate to phosphoenolpyruvate (34). Since the conversion of tricarboxylic acid (TCA) cycle intermediates to phosphoenolpyruvate is completely blocked in a ppsA pckA double mutant, this mutant is unable to grow on gluconeogenic nutrients, such as acetate, pyruvate, amino acids that feed into the TCA cycle, fatty acids, and TCA cycle intermediates, as sole carbon and energy sources but should be able to grow on phosphoenolpyruvate and amino acids that feed into the central carbon metabolism “above” pyruvate, as long as the wild-type strain can do so (35). Also, since glycolytic pathways are not affected in a ppsA pckA double mutant, this mutant should be able to grow as well as the wild type on sugars as sole carbon and energy sources (35). An E. coli Nissle 1917 ΔppsA ΔpckA mutant was constructed and, as expected, failed to grow on gluconeogenic nutrients but was still able to grow as well as wild-type E. coli Nissle 1917 on glycolytic nutrients (Table 2). Next, we tested the abilities of these strains to colonize mouse intestines.

When 10^6 CFU of E. coli Nissle 1917 and 10^7 CFU of E. coli Nissle 1917 ΔppsA ΔpckA were fed simultaneously to mice, by day 1 postfeeding, each had grown 2 orders of magnitude, suggesting that E. coli Nissle 1917 did not use gluconeogenic nutrients for growth in the intestine during that initial period, presumably because glycolytic nutrients were plentiful (Fig. 1). However, from

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Relative final tetrazolium dye reduction level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>0.81</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.00</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.65</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.81</td>
</tr>
</tbody>
</table>

TABLE 2 Biolog phenotypic analysis of E. coli Nissle 1917 strains grown on glycolytic and gluconeogenic carbon sources
day 1 to day 5 postfeeding, E. coli Nissle 1917 remained in the intestine in relatively constant numbers, whereas the numbers of E. coli Nissle 1917 ΔppsA ΔpckA bacteria dropped 2 orders of magnitude, suggesting that during that period, glycolytic nutrients were in short supply, forcing E. coli Nissle 1917 to switch, at least in part, to gluconeogenic nutrients, which E. coli Nissle 1917 ΔppsA ΔpckA was unable to do (Fig. 1). Since we already have established that E. coli Nissle 1917 uses glycolytic nutrients for colonization (14), these results also suggest that E. coli Nissle 1917 uses both glycolytic and gluconeogenic nutrients for growth in the mouse intestine between day 1 and day 5 postfeeding. Beyond day 5 postfeeding, the ratio of E. coli Nissle 1917 to E. coli Nissle 1917 ΔppsA ΔpckA remained fairly constant (Fig. 1), suggesting either that E. coli Nissle 1917 stopped using gluconeogenic nutrients for growth in the intestine or that E. coli Nissle 1917 ΔppsA ΔpckA maintained itself in constant numbers by switching to the use of one or more glycolytic nutrients not being used by E. coli Nissle 1917. Since there is no obvious reason why E. coli Nissle 1917 ΔppsA ΔpckA would use glycolytic nutrients not being used by E. coli Nissle 1917, we favor the hypothesis that beyond day 5 postfeeding, E. coli Nissle 1917 and E. coli Nissle 1917 ΔppsA ΔpckA colonized an intestinal niche in which glycolytic nutrients were in ample supply, so that the use of gluconeogenic nutrients was not necessary, and therefore, E. coli Nissle 1917 stopped using them for growth. For discussion purposes, we will assume that this is the case. In contrast to the 2- to 3-order-of-magnitude advantage that E. coli Nissle 1917 displayed in the intestine when 10⁵ CFU of wild-type E. coli Nissle 1917 and 10⁵ CFU of E. coli Nissle 1917 ΔppsA ΔpckA were fed simultaneously to mice, the colonization abilities of these strains were identical when they were fed separately, each colonizing at about 10⁶ CFU/g of feces (compare Fig. 3 and 6). Parenthetically, these data make it unlikely that growth or survival in feces has a differential effect on either strain and thereby validate the use of fecal counts to measure their relative colonizing abilities when both strains are fed to mice simultaneously.

E. coli EDL933 ΔppsA ΔpckA is nearly eliminated from the intestines of mice precolonized with E. coli Nissle 1917, but not with E. coli MG1655. Humans infected with E. coli O157:H7 strains are presumably colonized with at least one commensal E. coli strain prior to infection. To mimic that situation, streptomycin-treated mice can be precolonized with a commensal E. coli strain for 10 days and then fed E. coli EDL933 (14, 36). When mice were precolonized with the commensal E. coli strain MG1655 for 10 days and were then fed 10⁵ CFU each of wild-type E. coli EDL933 and E. coli EDL933 ΔppsA ΔpckA, wild-type E. coli EDL933 grew to a level between 10⁶ and 10⁷ CFU/g of feces, whereas E. coli EDL933 ΔppsA ΔpckA dropped to about 10⁴ CFU/g of feces (Fig. 2A), suggesting, as reported previously (15), that under these conditions, E. coli EDL933 uses both glycolytic and gluconeogenic nutrients for colonization. In contrast, in mice precolonized with E. coli MG1655 for 10 days and then fed 10⁵ CFU of E. coli EDL933 ΔppsA ΔpckA but no wild-type E. coli EDL933, the gluconeogenesis mutant maintained itself at a level between 10⁵ and 10⁶ CFU/g of feces (Fig. 2B), thereby validating the use of fecal counts to measure their relative colonization. In contrast, in mice precolonized with E. coli EDL933 ΔppsA ΔpckA but no wild-type E. coli EDL933, the gluconeogenesis mutant maintained itself at a level between 10⁵ and 10⁶ CFU/g of feces (Fig. 2B), thereby validating the use of fecal counts to measure their relative colonization.
and 10⁶ CFU/g of feces, 100-fold higher than the level in the presence of wild-type E. coli EDL933 (Fig. 2B), suggesting that in the absence of wild-type E. coli EDL933, enough glycolytic nutrients were available for E. coli EDL933 ΔppsA ΔpckA to sustain that level in the intestine. In similar experiments, when mice were precolonized with E. coli Nissle 1917 for 10 days and then fed 10⁵ CFU each of wild-type E.coli EDL933 and E. coli EDL933 ΔppsA ΔpckA, wild-type E. coli EDL933 grew to a level between 10⁵ and 10⁶ CFU/g of feces, whereas E. coli EDL933 ΔppsA ΔpckA was nearly eliminated, dropping to about 10⁵ CFU/g of feces (Fig. 3A). Moreover, in the absence of wild-type E. coli EDL933, E. coli EDL933 ΔppsA ΔpckA levels still dropped to about 10⁵ CFU/g of feces in mice precolonized with E. coli Nissle 1917 for 10 days (Fig. 3B), suggesting that in mice precolonized with E. coli Nissle 1917, invading E. coli EDL933 must use both the available glycolytic and gluconeogenic nutrients to maintain itself in the intestine; i.e., the available concentration of glycolytic nutrients alone is not sufficient. Furthermore, since in the absence of wild-type E. coli EDL933, E. coli EDL933 ΔppsA ΔpckA colonized mice precolonized with E. coli Nissle 1917 for 10 days at a level 1,000-fold lower than that for mice precolonized with E. coli MG1655 for 10 days (compare Fig. 2B and 3B), it appears that far fewer glycolytic nutrients were available to E. coli EDL933 in E. coli Nissle 1917-precolonized mice than in E. coli MG1655-precolonized mice.

In the absence of precolonization with any commensal E. coli strain, E. coli EDL933 and E. coli EDL933 ΔppsA ΔpckA fed to mice treated with streptomycin for 10 days colonize mouse intestines equally well. The data to this point suggested that precolonization of mouse intestines with E. coli Nissle 1917 leaves far fewer glycolytic nutrients available to invading E. coli EDL933 than does precolonization with E. coli MG1655, raising the possibility that E. coli EDL933 competes for glycolytic nutrients only/maintained by the preadministered E. coli strain and not with the rest of the microbiota. To determine whether E. coli EDL933 does or does not compete with the microbiota to colonize, mice were treated with streptomycin but were not precolonized with any E. coli strain. At day 10, the mice were fed 10⁵ CFU each of E. coli EDL933 and E. coli EDL933 ΔppsA ΔpckA. Indeed, the two strains grew at the same rate in mouse intestines for 3 days, to a level of about 10⁶ CFU/g of feces, and cocolonized intestines at a level of about 10⁸ CFU/g of feces thereafter (Fig. 4). These results suggest that in the absence of E. coli MG1655, E. coli Nissle 1917, or any other E. coli strain, glycolytic nutrients were in sufficient supply in the intestine such that a switchover to gluconeogenic nutrients by E. coli EDL933 was not necessary. Parenthetically, the results show that E. coli EDL933 and E. coli EDL933 ΔppsA ΔpckA grow/survive equally well in feces, thus validating the use of fecal plate counts in experiments comparing the two strains in mice.

Visualization of a dense and diverse microbiota in the cecal mucus of mice treated with streptomycin for 10 days. E. coli strains colonize the large intestines of streptomycin-treated mice by growing in the mucus layer as members of mixed biofilms (12, 13), and the glycolytic nutrients they use for growth appear to be mono- and disaccharides produced by the anaerobes as extracellular degradation products of oligo- and polysaccharides (14, 37,
Clearly, there were sufficient mono- and disaccharides in the intestines of mice fed streptomycin for 10 days in the absence of any precolonizing E. coli strain to allow E. coli EDL933 to grow to high numbers without using gluconeogenic nutrients (Fig. 4), suggesting that if a robust anaerobe population was present, it was producing mono- and disaccharides but not using them for growth. Indeed, the cecal mucus layer in mice fed streptomycin for 10 days in the absence of any precolonizing E. coli strain did contain a large and diverse microbiota consisting of members of various sizes and shapes, but no E. coli (Fig. 5A). As expected, E. coli was present in the cecal mucus of mice precolonized with E. coli Nissle 1917 for 10 days (Fig. 5B).

**Growth of E. coli EDL933 and E. coli EDL933 ΔppsA ΔpckA in the intestines of mice precolonized with E. coli Nissle 1917 ΔppsA ΔpckA.** The data presented in Fig. 1 suggested that E. coli Nissle 1917 uses gluconeogenic nutrients for growth in the mouse intestine between day 1 and day 5 postfeeding but then stops using them. However, when mice were precolonized with E. coli Nissle 1917 ΔppsA ΔpckA for 10 days and were then fed 10^7 CFU each of wild-type E. coli EDL933 and E. coli EDL933 ΔppsA ΔpckA, wild-type E. coli EDL933 grew to about 10^7 CFU/g of feces (Fig. 6A), a level >10-fold higher than that in mice precolonized with wild-type E. coli Nissle 1917 (P < 0.00001) (compare Fig. 3A and 6A, and see Materials and Methods for an explanation of P values), suggesting that E. coli Nissle 1917 does use gluconeogenic nutrients to limit the growth of E. coli EDL933 (Fig. 1). Moreover, E. coli EDL933 ΔppsA ΔpckA was nearly eliminated, dropping to about 10^2 CFU/g of feces (Fig. 6A), suggesting that E. coli EDL933 uses both glycolytic and gluconeogenic nutrients for growth in mice precolonized with E. coli Nissle 1917 ΔppsA ΔpckA.

We wanted to test further whether E. coli Nissle 1917 uses gluconeogenic nutrients to limit the growth of E. coli EDL933 in the mouse intestine, as suggested by the preceding experiment. If E. coli Nissle 1917 was not using gluconeogenesis to limit the growth of E. coli EDL933, precolonization with E. coli Nissle 1917 ΔppsA ΔpckA should limit colonization by E. coli EDL933 ΔppsA ΔpckA as well as precolonization with wild-type E. coli Nissle 1917 does. However, we found that E. coli EDL933 ΔppsA ΔpckA colonized the intestines of mice precolonized with E. coli Nissle 1917 ΔppsA ΔpckA at a level between 10^4 and 10^5 CFU/g of feces (Fig. 6B), >1,000-fold higher than the level in mice precolonized with E. coli Nissle 1917 (compare Fig. 3B and 6B). Therefore, it appears that E. coli Nissle 1917 does use gluconeogenic nutrients to nearly eliminate E. coli EDL933 ΔppsA ΔpckA from the mouse intestine, despite appearing to stop using gluconeogenic nutrients 5 days post-feeding (Fig. 1).

**DISCUSSION**

It was reported recently that E. coli 86-24, an O157:H7 strain, activates the expression of virulence genes under gluconeogenic conditions (16, 17). The present study was undertaken to determine whether E. coli Nissle 1917, routinely used as a probiotic agent, depletes gluconeogenic nutrients by using them for growth in the mouse intestine, thereby preventing E. coli EDL933, also an O157:H7 strain, from activating virulence gene expression. We found that E. coli Nissle 1917 does indeed use gluconeogenic nutrients to colonize the mouse intestine (Fig. 1). Yet we also found that E. coli EDL933 relies heavily on gluconeogenesis to colonize mice precolonized with E. coli Nissle 1917 for 10 days; i.e., under these conditions, wild-type E. coli EDL933 colonized at a level almost 10,000-fold higher than that of E. coli EDL933 ΔppsA ΔpckA at day 21 (Fig. 3A). These data eliminate the possibility that E. coli Nissle 1917 depletes the intestine of gluconeogenic nutrients and therefore show that E. coli Nissle 1917 would not prevent the activation of virulence gene expression by E. coli EDL933, assuming that virulence gene expression is activated in E. coli EDL933 as it is in E. coli 86-24. We also showed that only under conditions in which E. coli EDL933 competes for nutrients with precolonizing E. coli strains does it utilize gluconeogenic nutrients.
for growth in the mouse intestine; i.e., in mice that had not been precolonized with any *E. coli* strain, but that contained a dense and apparently diverse microbiota (Fig. 5A), wild-type *E. coli* EDL933 and *E. coli* EDL933 Δ*ppsA* Δ*pckA* grew at the same rate, to about 10⁵ CFU/g of feces, and cocolonized mice equally well thereafter, at about 10⁶ CFU/g of feces (Fig. 4).

Despite the finding that *E. coli* EDL933 switches in part to gluconeogenesis in both *E. coli* MG1655- and *E. coli* Nissle 1917-precolonized mice (Fig. 2 and 3), differences in the competition of *E. coli* EDL933 Δ*ppsA* Δ*pckA* against *E. coli* MG1655 and against *E. coli* Nissle 1917 were observed. That is, in mice precolonized for 10 days with *E. coli* Nissle 1917 and fed 10⁵ CFU of *E. coli* MG1655 Δ*ppsA* Δ*pckA*, the gluconeogenesis mutant was nearly eliminated, colonizing at a level of only 10⁵ CFU/g of feces (Fig. 3B). In contrast, in mice precolonized with *E. coli* MG1655 and fed 10⁵ CFU of *E. coli* EDL933 Δ*ppsA* Δ*pckA*, the gluconeogenesis mutant colonized at a level of 10⁶ CFU/g of feces, 1,000-fold higher than the level in mice precolonized with *E. coli* Nissle 1917 (Fig. 2B). Therefore, it appears that glycolytic nutrients were less available to *E. coli* EDL933 in mice precolonized with *E. coli* MG1655. In support of this view, *E. coli* Nissle 1917 colonized the intestine at a level about 10-fold higher than *E. coli* MG1655 (*P* < 0.00001) (compare Fig. 2 and 3) and presumably used more glycolytic nutrients in the process, leaving fewer glycolytic nutrients for *E. coli* EDL933.

We also found that *E. coli* Nissle 1917 uses gluconeogenic nutrients for growth in the intestines of streptomycin-treated mice between day 1 and day 5 postfeeding but appears to stop using them thereafter (Fig. 1). Paradoxically, we found that gluconeogenic nutrients are used by precolonizing *E. coli* Nissle 1917 at and beyond 10 days postfeeding to limit the growth of invading *E. coli* EDL933 (Fig. 3A and B). How, then, does precolonizing *E. coli* Nissle 1917 appear to stop using gluconeogenic nutrients that are available in the mouse intestine beyond 5 days postfeeding and yet use them to limit the growth of invading *E. coli* EDL933 at 10 days postfeeding? This paradox can be explained if beyond 5 days postfeeding, *E. coli* Nissle 1917 resides both in a large niche in the mouse intestine, in which it uses only glycolytic nutrients, and in a much smaller niche, where it competes with invading *E. coli* EDL933 for both glycolytic and gluconeogenic nutrients. This explanation fits well with our “restaurant” hypothesis, which we summarize immediately below and which we recently proposed to account for the fact that *E. coli* mutants that grow poorly relative to their parents in bacterium-free cecal mucus *in vitro* can colonize the mouse intestine better than their parents (12, 13).

It is known that to grow in and colonize the mouse large intestine, *E. coli* strains must penetrate into the mucus layer (10, 11). It is also known that *E. coli* strains reside in mucus as members of mixed biofilms (12, 13). Furthermore, *E. coli* strains use monosaccharides and disaccharides as glycolytic nutrients for growth in the mouse large intestine (14, 37), yet mono- and disaccharides are absorbed in the small intestine. Since most *E. coli* strains do not secrete extracellular polysaccharide hydrolases (38–40) and therefore cannot degrade dietary-fiber-derived and mucin-derived oligo- and polysaccharides, it is likely that during long-term colonization, *E. coli* depends on the anaerobes in mucus that can degrade oligo- and polysaccharides, *e.g.*, *Bacteroides thetaiotaomicron* (41), to provide them with the mono- and disaccharides they use for growth. We hypothesized (13) that during the initiation phase of large-intestine colonization, a commensal *E. coli* strain enters an initiation niche in mucus in which it competes for nutrients from a perfectly mixed pool with any other *E. coli* strain present in that niche. We propose that it is in this niche that *E. coli* Nissle 1917 is a better colonizer than *E. coli* Nissle 1917 Δ*ppsA* Δ*pckA* between day 1 and day 5 postfeeding, because *E. coli* Nissle 1917 is able to use both glycolytic and gluconeogenic nutrients for growth, and it is in this niche that *E. coli* Nissle 1917 competes with invading *E. coli* EDL933 for both glycolytic and gluconeogenic nutrients. We also hypothesized that in addition to continuously colonizing the initiation niche during long-term colonization, each commensal *E. coli* strain also resides on a long-term basis as a member of mixed biofilms we see in mucus (12, 13), together with specific anaerobes. We called the mixed biofilms “restaurants,” and we hypothesized that each commensal *E. coli* strain receives monosaccharides locally from specific anaerobes with which it interacts physically and metabolically rather than from a perfectly mixed pool of nutrients and as such does not compete directly with any other *E. coli* strain within its “restaurant.” We hypothesize that these biofilms, presumably formed by 5 days postfeeding, constitute the larger niche, which is colonized equally well by *E. coli* Nissle 1917 and *E. coli* Nissle 1917 Δ*ppsA* Δ*pckA*, because sufficient glycolytic nutrients are made available locally by the anaerobes residing there such that any gluconeogenic nutrients

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**FIG 6** Colonization of the intestines of streptomycin-treated mice by preadministered *E. coli* Nissle 1917 Δ*ppsA* Δ*pckA*, *E. coli* EDL933, and *E. coli* EDL933 Δ*ppsA* Δ*pckA*. (A) Three sets of 3 mice were fed 10⁵ CFU of *E. coli* Nissle 1917 Δ*ppsA* Δ*pckA* on day 0 (●) and 10⁵ CFU each of *E. coli* EDL933 ΔpckA (■) and *E. coli* EDL933 Δ*ppsA* Δ*pckA* (▲) on day 10 postfeeding. (B) Two sets of 3 mice were fed 10⁵ CFU of *E. coli* MG1655 Δ*ppsA* Δ*pckA* on day 0 (●) and 10⁵ CFU of *E. coli* EDL933 Δ*ppsA* Δ*pckA* (▲) on day 10 postfeeding. At the indicated times, fecal samples were homogenized, diluted, and plated as described in Materials and Methods. Data are mean log₁₀ CFU per gram of feces for each time point; error bars represent standard errors.
produced are not needed by *E. coli* Nissle 1917 for growth and therefore diffuse to the smaller initiation niche.

Different *E. coli* strains use different glycolytic nutrients to colonize the intestines of streptomycin-treated mice (14, 37). We recently reported that when mice were precolonized with *E. coli* HS, a human commensal strain, and *E. coli* Nissle 1917, which together use 5 of 7 known glycolytic nutrients used by *E. coli* EDL933 to colonize (14), invading *E. coli* EDL933 bacteria were completely eliminated from the mouse intestine (14). However, precolonizing *E. coli* HS and *E. coli* Nissle 1917 were unable to eliminate *E. coli* CFT073, a uropathogenic *E. coli* (UPEC) strain, or *E. coli* E2348/69, an enteropathogenic *E. coli* (EPEC) strain (14). In fact, both pathogens grew to levels of about 10^8 CFU/g of feces under these conditions (14). Although the possibility exists that *E. coli* CFT073 and *E. coli* E2348/69 use several glycolytic nutrients that *E. coli* HS and *E. coli* Nissle 1917 do not use to colonize the mouse intestine, thus allowing their growth, the data presented here raise the possibility that different *E. coli* pathogens may also use different gluconegenic nutrients that contribute to their ability to colonize in the face of competition from commensal strains. Further research is required to test this possibility.

ACKNOWLEDGMENT
This research was supported by Public Health Service grant GM095370 to T.C. and P.S.C.

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