INFECTION AND IMMUNITY, Apr. 2007, p. 1661–1666 Vol. 75, No. 4
0019-9567/07/$08.00+0 doi:10.1128/IAI.01342-06

Enterohemorrhagic *Escherichia coli* O157:H7 *gal* Mutants Are Sensitive to Bacteriophage P1 and Defective in Intestinal Colonization▽

Theresa Deland Ho and Matthew K. Waldor*

Department of Molecular Biology and Microbiology, Tufts University School of Medicine and Howard Hughes Medical Institute, 136 Harrison Avenue, Boston, Massachusetts 02111

Received 21 August 2006/Returned for modification 20 October 2006/Accepted 9 November 2006

Enterohemorrhagic *Escherichia coli* (EHEC), especially *E. coli* O157:H7, is an emerging cause of food-borne illness. Unfortunately, *E. coli* O157 cannot be genetically manipulated using the generalized transducing phage P1, presumably because its extensive O antigen obscures the P1 receptor, the lipopolysaccharide (LPS) core subunit. The GalE, GalT, GalK, and GalU proteins are necessary for modifying galactose before it can be assembled into the repeating subunit of the O antigen. Here, we constructed *E. coli* O157:H7 *gal* mutants which presumably have little or no O antigen. These strains were able to adsorb P1. P1 lysates grown on the *gal* mutant strains could be used to move chromosomal markers between EHEC strains, thereby facilitating genetic manipulation of *E. coli* O157:H7. The *gal* mutants could easily be reverted to a wild-type Gal$^+$ strain using P1 transduction. We found that the O157:H7 *galETKM*::*aad-7* deletion strain was 500-fold less able to colonize the infant rabbit intestine than the isogenic Gal$^+$ parent, although it displayed no growth defect in vitro. Furthermore, in vivo a Gal$^+$ revertant of this mutant outcompeted the *galETKM* deletion strain to an extent similar to that of the wild type. This suggests that the O157 O antigen is an important intestinal colonization factor. Compared to the wild type, EHEC *gal* mutants were 100-fold more sensitive to a peptide derived from bactericidal permeability-increasing protein, a bactericidal protein found on the surface of intestinal epithelial cells. Thus, one way in which the O157 O antigen may contribute to EHEC intestinal colonization is to promote resistance to host-derived antimicrobial polypeptides.

Enterohemorrhagic *Escherichia coli* (EHEC) is an emerging cause of food-borne illness. After ingestion of contaminated food, humans can develop symptoms ranging from mild diarrhea to the severe, and at times life-threatening, condition hemolytic-uremic syndrome. Currently, EHEC is the most common cause of pediatric renal failure in the United States (15). Besides causing significant human disease, EHEC is a major economic burden due to the high cost associated with the recall of contaminated beef (www.fsis.usda.gov/FSIS_Recalls/index.asp). Several EHEC serotypes cause disease, but the O157 serotype is by far the most common cause of EHEC-related disease in North America, Europe, and Japan (6).

Lipopolysaccharide (LPS), found in the outer membrane of gram-negative bacteria, is composed of lipid A, core oligosaccharide, and repeating O-antigen subunits. The O antigen is covalently linked to the outer region of the core oligosaccharide, and it appears to act as a barrier that can protect enteric pathogens against toxic agents encountered in host gastrointestinal tracts (23). For example, *galU* and *galE* mutants of *Vibrio cholerae*, which lacked O antigen, were defective in intestinal colonization, although they had no growth defect in rich medium. These mutants were more sensitive than O-antigen-producing strains to killing by complement and cationic antimicrobial peptides, suggesting that their defect in colonization was attributable to their sensitivity to bactericidal substances elaborated by the host gastrointestinal tract (17).

Like many enteric pathogens, *E. coli* O157 produces LPS that contains an extensive O antigen. The O157 O-antigen subunit consists of *N*-acetyl-D-perosamine, L-fucose, D-glucose, and *N*-acetyl-D-galactose (22). Production of *N*-acetyl-D-galactose requires that its precursor, galactose, be modified by the enzymes GalE, GalT, GalK, and GalU. *Salmonella enterica* serovar Typhimurium and *E. coli gal* mutants do not make O antigen (8, 18, 25).

The inner region of the LPS core oligosaccharide, which is conserved in many enteric bacteria, serves as the receptor for bacteriophage P1. Phage P1 has been a workhorse for genetic manipulation of *E. coli* K-12 for many decades. P1-mediated generalized transduction enables movement of mutations for generation of isogenic bacterial strains, which is often required for proving the linkage between particular genotypes and phenotypes. In *S. enterica* serovar Typhimurium, which has an LPS core oligosaccharide similar to that of *E. coli* K-12, the long O antigen obscures the core oligosaccharide and prevents P1 from adsorbing to the bacteria. O-antigen mutants (Δ*gal*, Δ*galE*, and Δ*galU*) of *S. enterica* serovar Typhimurium have been shown to be P1 sensitive (18).

P1-mediated generalized transduction of EHEC has not been described previously. Here, we adopted the strategy that was used to facilitate P1 transduction in *Salmonella*. Wild-type EHEC O157:H7 strains are resistant to P1, but O157:H7 *gal* mutants were found to be P1 sensitive and permitted P1-mediated movement of genetic markers between EHEC strains. Furthermore, we developed a method that allows a simple reversion to convert P1-sensitive strains to the wild type. Interestingly, we found that the P1-sensitive *galETKM* O157:H7

* Corresponding author. Present address: Channing Laboratories, Brigham and Women's Hospital, 181 Longwood Ave., Boston, MA 02115. Phone: (617) 525-4650. Fax: (617) 525-4660. E-mail: mwaldor@rics.bwh.harvard.edu.

▽ Published ahead of print on 11 December 2006.

TABLE 1. Strains used in this study

Strain	Genotype or description	Reference or source
EDL933	O157:H7	21
TEA007	EDL933 *galE*::pTHE001	This study
TEA023	EDL933 Δ*galU*::*aad-7*	This study
TEA026	EDL933 Δ*galETKM*::*aad-7*	This study
TEA028	EDL933 Δ*galETKM*::*tetA*	This study
TEA040	EDL933; Gal$^+$ revertant of TEA026	This study
CAG5051	HfrH *nadA57*::Tn*10 thi-1 relA1 spoT supQ80*	28
MC4100	*araD139* Δ(*arg-lac*)*U169 rpsL150 relA1 flbB5301 fruA25 deoC1 ptsF25*	Laboratory stock
EDL933/pKD46	EDL933/pKD46 (oriR101 *bla* Para-λ *gam bet exo*)	This study
BW19851/pGP704	RP-4-2-*Tc*::Mu-1 *kan*::Tn*7* integrant; *creB150 hsdR17 endA zbf*-5 *uidA*(ΔMlu1)::*pir*(wt) *recA1 thi*/pGP704 (oriR6K *mob*RP4 Ap^r)	16
WM3064/pTHE001	*thrB1004 pro thi rpsL hsdS lacZ*ΔM15 RP4-1360 Δ(*araBAD*)*567* Δ*dapA1341*::[*erm pir*(wt)]/pTHE001 (oriR6K *mob*RP4 Ap^r *galE*$_{EDL933}$)	This study
DH5αλ*pir*$^+$/pVi36	*endA1 hadR17 thi-1 recA1* Δ(*lacIZYA-argF*)*U169 deoR* (*p80 lacZ*ΔM15) *gyrA relA1*/pVi36 (oriR6K *aad-7*)	V. Burrus
BW23473/pAH162	Δ(*lacIZYA-argF*)*U169 rph-1 rpoS396*(Am) *robA1 creC510 hsdR514* Δ*endA95 uidA*(ΔMlu1)::*pir*(wt) *recA1*/pAH162 (*tetA*)	11

mutant was extremely attenuated in the ability to colonize the infant rabbit intestine and had increased sensitivity to bactericidal permeability-increasing protein (BPI).

MATERIALS AND METHODS

Bacterial strains and growth. The strains used in this study are shown in Table 1. Unless indicated otherwise, strains were grown in LB broth or on LB agar plates. For antibiotic selection, agar plates were supplemented with ampicillin (80 μg/ml), spectinomycin (100 μg/ml), or tetracycline (6 μg/ml). MacConkey agar plates with 1% galactose or M63 [22 mM KH_2PO_4, 40 mM K_2HPO_4, 15 mM $(NH_4)_2SO_4$, 0.5 mg/liter $FeSO_4$] agar plates supplemented with 0.2% galactose and 0.1% Casamino Acids were used to test whether a strain could metabolize galactose.

To generate the deletion-insertion mutations in the *gal* genes, we used a one-step gene inactivation method adapted from the method of Datsenko and Wanner (5). In this method, a temperature-sensitive plasmid (pKD46) encoding lambda red recombinase was transformed into EDL933. To make the Δ*galU*::*aad-7* mutant TEA023 and the Δ*galETKM*::*aad-7* mutant TEA026, the spectinomycin resistance gene (*aad-7*) was amplified from the pVi36 plasmid (provided by V. Burrus, University of Sherbrooke) template using primers TE139 (5′-ATGGCTGCCATTAATACGAAAGTCAAAAAAGCC) and TE140 (5′-TTACTTCTTAATGCCCATCTCTTCTTCAAGCCA) and primers TE137 (5′-ATGCTATGGTTATTTCATACCATAAGCCTAATGGAGCCCGGCGGATTTGTCCTACTC) and TE138 (5′-TTACTCAGCAATAAACTGATATTCCGTCAGGCTCTAAGCACTTGTCTCCTGTTTA), respectively. To obtain Δ*galETKM*::*tetA* mutant TEA028, the tetracycline resistance gene (*tetA*) was amplified from the pAH162 plasmid (11) template using primers TE141 (5′-ATGCTATGGTTATTTCATACCATAAGCCTAATGGAGGATGCCTGGCAGTTCCCTACTC) and TE142 (5′-TTACTCAGCAATAAACTGATATTCCGTCAGGCTTTAGGTGGCGGTACTTGGGTCGA). After electroporation of the PCR products, cells were incubated in SOB (Invitrogen) containing 0.2% L-arabinose for 2 h and then plated on selective media at 37°C. For the Δ*galU*::*aad-7* mutation, the spectinomycin resistance gene replaced all of the *galU* gene except the first 33 bp and the last 30 bp of the *galU* open reading frame. For the Δ*galETKM*::*aad-7* and Δ*galETKM*::*tetA* mutations, the antibiotic resistance gene replaced all of the *galETKM* operon except the first 36 bp of the *galE* gene and the last 30 bp of the *galM* gene.

We constructed the pTHE001 plasmid to generate an insertion mutation in *galE*. First, a 460-bp internal fragment of the *galE* gene was amplified by PCR using primers TE013 (5′-GCAAGGATCCGACGTTTGTTGAAGGCGATA) and TE014 (5′-GGCATAAGGGAATTCGGAATGCCTTGCGGA). The PCR product was digested with BamHI and then cloned into the BglII site of the conditional plasmid pGP704 (16). The resulting plasmid, pTHE001, was mobilized using the RP4$^+$ helper strain WM3064 (provided by W. Metcalf, University of Illinois, Urbana-Champaign) into EDL933.

To generate the Gal$^+$ revertant TEA040, we first moved the *galE*::pTHE001 mutation from TEA007 into the Δ*galETKM*::*aad-7* strain (TEA026) by P1 transduction, selecting for ampicillin resistance. The resulting strain was then used as a recipient for P1 transduction of the *galETKM*$^+$ allele from TEA023. Gal$^+$ transductants were selected on M63 agar plates supplemented with 0.2% galactose and 0.1% Casamino Acids.

P1 adsorption and sensitivity assays. P1 adsorption assays were performed using a P1 lysate grown on *E. coli* K-12 strain MC4100. Approximately 100 μl of an overnight culture was pelleted by centrifugation and resuspended in 100 μl of LB broth. Then 100 μl of the P1 lysate was added to the cells. After 15 min of incubation at 37°C, cells were pelleted by centrifugation at 6,000 rpm in an Eppendorf centrifuge at 4°C for 2 min. The P1 titers of the supernatants were then determined by plaquing, using MC4100 as an indicator strain. To plaque P1, we spotted phage lysates on top agar (LB broth containing 2 mM $MgSO_4$, 10 mM $CaCl_2$, and 0.7% agar) lawns of MC4100.

To test for sensitivity of strains to P1 lysis, each strain was cross-streaked against P1. A single line of P1 (100 μl; ~10^9 PFU/ml) was allowed to dry on an LB agar plate. For each bacterial strain, a single streak was then drawn perpendicular to a line of phage P1. Strains resistant to P1 grew on both sides of the line of P1; susceptible strains were partially lysed following an encounter with P1.

P1 lysate production. P1 lysates of various EHEC strains were generated by growing 1:100 dilutions of overnight cultures of each strain in 2.5 ml of LB broth containing 2 mM $MgSO_4$ and 10 mM $CaCl_2$ and incubating the preparations for 1 h at 37°C with agitation. Then 100 μl of a P1 lysate grown on MC4100 was added to each culture. After 2 to 3 h of incubation at 37°C with agitation, lysis of the cultures was observed. Tubes were transferred to ice, and any remaining intact bacteria were lysed with 0.5 ml chloroform. Lysates were then centrifuged at 13,000 rpm for 1 min, diluted in phosphate-buffered saline (PBS), and spotted on top agar lawns of MC4100 for titration. Lysates were stored at 4°C in the dark with 0.5 ml chloroform.

P1 transduction. Overnight cultures (0.5 ml) of recipient bacteria grown in LB broth were pelleted and resuspended in 100 μl MC (5 mM $MgSO_4$, 50 mM $CaCl_2$). About 50 μl of P1 lysate was added to the cells, which were then incubated at 37°C for 15 to 30 min. LB broth with 10 mM sodium citrate (0.5 to 1 ml) was added to each tube and incubated for 1 h at room temperature. Each tube was centrifuged at 6,000 rpm for 2 min, and the pellets were resuspended in 100 μl 1 M sodium citrate and plated on selective media.

In vitro competition assays. A 1:1 mixture of a mutant (TEA026) and wild-type strain EDL933 or TEA040 initially containing 5×10^7 bacteria/ml was incubated in LB broth at 37°C with agitation overnight. Each assay mixture was then diluted and plated on LB agar. After overnight growth, bacteria were replica plated on selective media to determine the numbers of mutant and wild-type bacteria. Each assay was performed at least three times.

Competition assays with infant rabbits. We used the infant rabbit model to test the colonization ability of EHEC strains (27). Three-day-old New Zealand White rabbits were orally inoculated with a 1:1 mixture of TEA026 and wild-type strain EDL933 or TEA040 containing 2.5×10^8 bacteria which were washed one time and resuspended in PBS. Seven days after inoculation, rabbits were sacrificed, and their gastrointestinal tracts were removed. Portions of the ileum,

midcolon, and cecum were then homogenized, diluted, and plated on MacConkey agar containing sorbitol. After overnight growth, bacteria were replica plated on LB agar containing spectinomycin (100 μg/ml) to determine the number of TEA026 bacteria.

BPI sensitivity assays. We used the BPI-derived peptide P2, which has the antibacterial activity of whole protein (10, 13), to assess EHEC sensitivity. For these assays, bacteria grown overnight in LB broth were washed once in PBS and resuspended in PBS (pH 6.2). Then 5×10^7 bacteria with or without 30 μg P2 (Tufts University Core Facility) were incubated in 0.5 ml PBS (pH 6.2) for 45 min at 37°C. After incubation, each assay mixture was placed on ice, diluted, and plated on LB agar. Each assay was performed in triplicate and repeated in three independent experiments.

RESULTS AND DISCUSSION

O157:H7 *gal* mutants can adsorb P1 and are sensitive to P1 lysis. In many enteric bacteria, extensive LPS O antigens obscure the LPS core oligosaccharide and thereby prevent adsorption of and lysis by phage P1. Since synthesis of the O157:H7 O antigen requires modification of galactose by the *galE*, *galT*, *galK*, and *galU* gene products, we hypothesized that EHEC strains with mutations in these genes would produce no O antigen and therefore would be far more susceptible to P1 infection. To test this hypothesis, we compared phage infection of and adsorption by the sequenced O157:H7 isolate EDL933 to those of four *gal* derivatives: the Δ*galETKM*::*aad-7* (TEA026), Δ*galETKM*::*tetA* (TEA028), and Δ*galU*::*aad-7* (TEA023) deletion mutants and the *galE*::pTHE001 (TEA007) insertion mutant.

To assess whether *gal* loci influence infection of EHEC by phage P1, we performed cross-streak experiments. A streak of each bacterial strain was drawn perpendicular to a line of P1, and the consequences of encountering phage were assessed. There was no change in the growth of wild-type strain EDL933 in response to P1, indicating that this strain is resistant to phage infection. In contrast, all four *gal* mutants were lysed by phage P1 (data not shown).

To explore why the *gal* mutants are more susceptible to P1 infection, we examined whether the mutants showed enhanced adsorption of P1. Phage adsorption to the host bacterium is an essential step in phage infection. In these assays, each *gal* mutant was incubated with P1 and then centrifuged to pellet any phage adsorbed to the bacteria. Supernatants were then assayed to determine the number of unadsorbed P1 particles. As shown in Table 2, the wild-type strain adsorbed ~30% of the phage, suggesting that there may be some nonspecific interactions between EHEC and P1, since the wild-type strain is resistant to P1 infection and therefore is presumed to have inaccessible core oligosaccharides. However, all four *gal* mutants adsorbed ~95% of the P1, which is consistent with the hypothesis that access to P1's receptor is less impeded in these strains.

Antibiotic resistance markers can be transduced between EHEC O157:H7 *gal* mutants by P1. We next assessed whether EHEC *gal* mutants could serve as recipients or donors in P1 transduction. As expected, a *nadA*::Tn*10* marker from an *E. coli* K-12 strain (CAG5051) could not be transduced into the wild-type EDL933 strain by P1. In contrast, this marker could be transduced into the Δ*galU*::*aad-7* (TEA023), Δ*galETKM*::*aad-7* (TEA026), and *galE*::pTHE001 (TEA007) strains. To determine if chromosomal markers could be transduced from the EHEC *gal* mutants, each of the mutants was used to generate a P1 lysate. The Δ*galU*::*aad-7*, Δ*galETKM*::*aad-7*, and *galE*::pTHE001 mutations could be transferred readily into the O157:H7 Δ*galETKM*::*tetA* strain or into *E. coli* K-12 strain MC4100. For each of these EHEC P1 transduction experiments, between 10 and 100 transductants were obtained. Clearly, O157:H7 *gal* mutants can be transduced by P1 and can be used to generate P1 lysates. However, the frequencies of transduction between the O157:H7 *gal* strains were ~100-fold lower than the frequency of P1 transduction between K-12 strains. The lower O157:H7 transduction efficiency could be due to the abundance of prophage genes in O157:H7 that may affect replication or production of P1.

TABLE 2. P1 adsorption and P2 sensitivity of EHEC strains

Strain	% Adsorbed P1[a]	% Survival after P2 challenge[b]
EDL933 (wild type)	27.4	38
TEA007 (*galE*::pTHE001)	93.5	0.054
TEA023 (Δ*galU*::*aad-7*)	96.6	0.29
TEA026 (Δ*galETKM*::*aad-7*)	95.2	0.014
TEA028 (Δ*galETKM*::*tetA*)	98.4	0.35
TEA040 (Gal^+ revertant)	15.6	32

[a] The percentage of adsorbed P1 was determined as follows: (number of P1 plaques obtained after incubation with bacteria/number of P1 plaques obtained after incubation without bacteria) × 100%. Each experiment was performed in triplicate, and the data are means. The *P* value for a comparison of each of the *gal* mutants and the wild type was less than 0.006. The *P* value for a comparison of TEA040 and the wild type was 0.65.

[b] The percentage of survival after P2 challenge was determined as follows: (number of bacteria after incubation with P2/number of bacteria after incubation without P2) × 100%. Each experiment was performed in triplicate, and the data are means. The *P* value for a comparison of each of the *gal* mutants and the wild type was less than 0.03. The *P* value for a comparison of TEA040 and the wild type was 0.10.

Reversion of the *gal* mutant using P1 transduction. We devised a simple strategy to change Gal^- strains back to Gal^+ strains in order to enable study of Gal^+ EHEC strains that have been engineered using P1. We were unable to revert the Δ*galETKM*::*aad-7* mutant (TEA026) by transducing the *galETKM*$^+$ allele from TEA023, perhaps because the frequency of transduction was too low to obtain a revertant. Therefore, we utilized a two-step reversion method. We first moved the *galE*::pTHE001 mutation from TEA007 into the *galETKM* strain (TEA026). This strain was then used as a recipient for P1 transduction of the *galETKM*$^+$ allele from a donor lysate grown on the Δ*galU* strain (TEA023). Gal^+ transductants were selected on agar plates containing M63 minimal medium supplemented with 0.2% galactose and 0.1% Casamino Acids and were verified to be Gal^+ strains on MacConkey agar containing 1% galactose. This transduction was as efficient as transfer of other EHEC chromosomal markers. We chose one Gal^+ revertant strain (TEA040) to test for P1 sensitivity. Like the isogenic wild-type strain, this Gal^+ revertant had a reduced capacity to adsorb P1 phage compared to the *gal* mutants (Table 2), was resistant to P1 lysis in a P1 cross-streak experiment, and was not able to serve as a recipient for P1 transduction (data not shown).

O157:H7 *galETKM* mutant is dramatically impaired in colonization of the infant rabbit intestine. Previous studies have shown that *galE* mutants of *S. enterica* serovars are defective in intestinal colonization (9, 12). We were interested in determining whether the EHEC *galETKM* deletion resulted in a similar defect in intestinal colonization. To do this, we tested the *galETKM*::*aad-7* deletion mutant (TEA026) in a competition

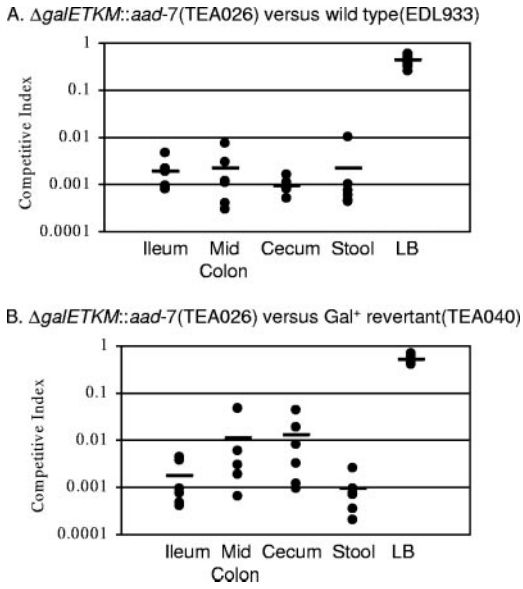

FIG. 1. In vivo and in vitro competition assays with an EHEC *galETKM* mutant (TEA026) and wild-type strain EDL933 or with TEA026 and a Gal$^+$ revertant of TEA026 (TEA040). Equal numbers of TEA026 and EDL933 (A) or TEA026 and TEA040 (B) were coinoculated into infant rabbits or into LB broth. After 7 days of infection, sections of the ileum, midcolon, and cecum and samples of stools were processed to determine competitive indices. The in vitro competition assays in LB broth were carried out for 16 h. The competitive index was determined as follows: [(number of output mutant bacteria)/(number of output wild-type or revertant bacteria)]/[(number of input mutant bacteria)/(number of input wild-type or revertant bacteria)]. Each of the competitive indices shown is statistically different from 1 ($P < 0.00001$, as determined by Student's *t* test).

assay with the isogenic wild-type strain (EDL933) using the EHEC-infant rabbit model. We found that the *galETKM* deletion mutant (TEA026) was ~500-fold less able to colonize the infant rabbit ileum, cecum, and midcolon (Fig. 1A). To demonstrate that this dramatic defect in intestinal colonization was due to the *galETKM* deletion, we performed a competition experiment with the Gal$^+$ reverted strain and its Δ*galETKM*::*aad-7* parent strain. The Gal$^+$ revertant (TEA040) outcompeted the *galETKM* mutant (TEA023) to an extent similar to the extent observed for the wild type (Fig. 1B), strongly suggesting that the *galETKM* deletion accounts for the colonization defect of TEA026. In vitro competition assays in which the *galETKM* strain and the wild type or the Gal$^+$ revertant were grown in LB broth at 37°C revealed that the *galETKM* mutation resulted in a slight (~2-fold) but statistically significant growth defect in rich medium (Fig. 1). This minor in vitro growth defect could not account for the drastic colonization defect of the *gal* mutant. Overall, these findings suggest that the O157 O antigen is critical for EHEC intestinal colonization.

O157:H7 *gal* mutants are more sensitive to BPI killing. Host organisms often produce antimicrobial peptides, such as cryptidins, as effectors of innate immunity. BPI is a 55- to 60-kDa protein that is found in the blood and on surfaces of epithelial cells throughout the gastrointestinal tract (2). BPI binds the lipid A component of LPS and has potent bactericidal activity against gram-negative bacteria (7). Given that O157 *gal* mutants likely lack O antigen, we hypothesized that these mutants would be more sensitive to BPI than the wild-type strain, since the lipid A portion of the *gal* strains' LPS would be more accessible to BPI binding. We used P2, a peptide that contains BPI residues 86 to 104 and that has the same cytotoxic activity as the entire protein, to test if the O157:H7 *gal* mutants had increased susceptibility to BPI. Bacteria were incubated in the presence or absence of P2, and then the number of bacteria was determined. Each of the *gal* mutants was far more sensitive to P2 than the wild-type EHEC strain or the Gal$^+$ revertant (Table 2). These data suggest that O157 O antigen is important for EHEC resistance to BPI.

Conclusions. EHEC strains, especially *E. coli* O157 strains, are significant causes of disease in many developed nations, yet there are no methods for transducing markers in this important group of pathogenic *E. coli*. It has been known for a long time that *S. enterica* serovar Typhimurium *gal* mutants have little or no O antigen, which makes these strains sensitive to P1 (18). Our findings strongly suggest that the O157 O antigen obscures the EHEC core oligosaccharide, the P1 receptor, in a similar fashion, since we found that O157:H7 *gal* mutants are sensitive to P1 infection. We also developed a relatively simple P1 transduction-based means to revert *gal* mutants to a Gal$^+$ phenotype. Although we describe a method to genetically manipulate O157:H7 with P1, this technique can likely be adapted for use with other EHEC serotypes and probably with other pathogenic *E. coli* strains (such as enteropathogenic *E. coli* and uropathogenic *E. coli*) as well. Figure 2 outlines a general scheme for using P1 transduction for genetic manipulation of EHEC. This figure shows the method that our lab routinely uses to generate isogenic EHEC strains. This ability to move chromosomal markers enables generation of isogenic strains containing single or multiple mutations. Given the relative ease of the P1 transduction technique outlined here, it should be possible to backcross mutations generated with the lambda red system into genetically identical backgrounds.

Our observations revealed that the O157 O antigen is an important EHEC colonization factor. Tissue culture experiments have suggested that the O157 O antigen is not likely to be involved in EHEC adherence to intestinal epithelial cells and may reduce EHEC type 3 secretion of effectors that promote adherence (1, 4, 19). The sensitivity of the O157 *gal* mutants to BPI supports the idea that at least part of the colonization defect of the *galETKM* mutant is explained by its reduced resistance to host antimicrobial factors. Although O157 strains are most commonly associated with EHEC-related illness in North America, Europe, and Japan, other EHEC serotypes (O26, O91, O103, and O111) can cause significant human disease (20). It will be interesting to assess whether O antigens from different EHEC serotypes provide equal resistance to host killing and act as effectively as the O157 O antigen in intestinal colonization. In fact, differences in the LPSs of the O1, O7, and O8 serotypes of K1 *E. coli* correlate with differences in the virulence of these serotypes (24). The P1 transduction method described here should facilitate the construction of isogenic strains which vary only in their O antigens, thus enabling exploration of the role of particular O antigens in EHEC virulence.

O antigens likely act as important barriers against extracellular assaults mounted by the host immune system. O-antigen

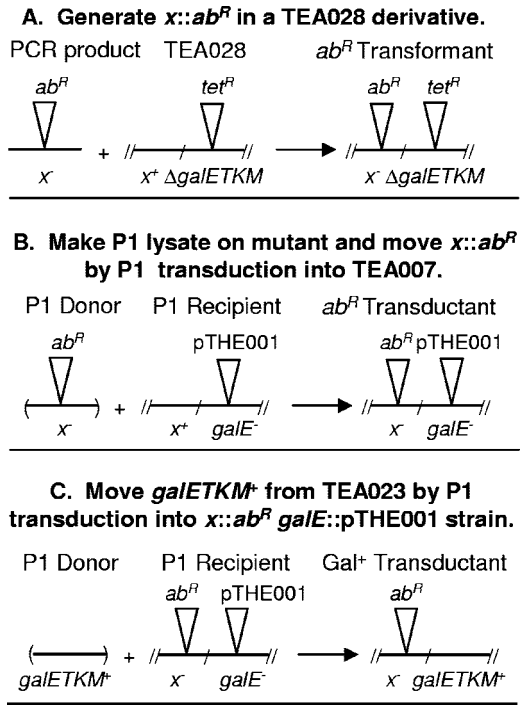

FIG. 2. General scheme for P1-facilitated genetic engineering of EHEC. (A) The desired mutation in the Δ*galETKM*::*tetA* or Δ*galETKM*::*aad-7* background is generated (e.g., using the lambda red recombination system) so that the mutation can be moved by P1 transduction. (B) The desired mutation is moved into the *galE*::pTHE001 background using P1 transduction, selecting for the antibiotic resistance gene to generate an isogenic backcrossed mutant. (C) A second P1 transduction into the backcrossed strain is performed, selecting for growth on galactose minimal plates. Abbreviations: *x*, gene of interest; ab^R, antibiotic resistance gene; tet^R, tetracycline resistance gene.

mutants of enteric bacteria like *V. cholerae*, *Klebsiella* serotype O1:K20, and *Shigella flexneri* are more susceptible to antimicrobial peptides and complement killing (14, 17, 29). *S. enterica* serovar Typhi, *Brucella melitensis*, and *V. cholerae* O-antigen mutants have been shown to be impaired in intestinal colonization as well (3, 9, 17, 26). In fact, the only oral live attenuated vaccine against *S. enterica* serovar Typhi used in the United States is a *galE* mutant. Given the pronounced attenuation of the O157:H7 Δ*galETKM*::*aad-7* mutant, perhaps EHEC *gal* mutants could also be developed into useful vaccines.

ACKNOWLEDGMENTS

We thank James Slauch for helpful comments and Brigid Davis and Craig D. Ellermeier for critical reading of the manuscript.

We thank the NIH and Howard Hughes Medical Institute for funding this work.

REFERENCES

1. **Bilge, S. S., J. C. Vary, Jr., S. F. Dowell, and P. I. Tarr.** 1996. Role of the *Escherichia coli* O157:H7 O side chain in adherence and analysis of an *rfb* locus. Infect. Immun. **64:**4795–4801.
2. **Canny, G., O. Levy, G. T. Furuta, S. Narravula-Alipati, R. B. Sisson, C. N. Serhan, and S. P. Colgan.** 2002. Lipid mediator-induced expression of bactericidal/permeability-increasing protein (BPI) in human mucosal epithelia. Proc. Natl. Acad. Sci. USA. **99:**3902–3907.
3. **Chiang, S. L., and J. J. Mekalanos.** 1999. *rfb* mutations in *Vibrio cholerae* do not affect surface production of toxin-coregulated pili but still inhibit intestinal colonization. Infect. Immun. **67:**976–980.
4. **Cockerill, F. III, G. Beebakhee, R. Soni, and P. Sherman.** 1996. Polysaccharide side chains are not required for attaching and effacing adhesion of *Escherichia coli* O157:H7. Infect. Immun. **64:**3196–3200.
5. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
6. **Feng, P.** 2001. *Escherichia coli*, p. 143–162. *In* R. G. Labbe and S. Garcia (ed.), Guide to foodborne pathogens. John Wiley and Sons, Inc., New York, NY.
7. **Gazzano-Santoro, H., J. B. Parent, L. Grinna, A. Horwitz, T. Parsons, G. Theofan, P. Elsbach, J. Weiss, and P. J. Conlon.** 1992. High-affinity binding of the bactericidal/permeability-increasing protein and a recombinant amino-terminal fragment to the lipid A region of lipopolysaccharide. Infect. Immun. **60:**4754–4761.
8. **Genevaux, P., P. Bauda, M. S. DuBow, and B. Oudega.** 1999. Identification of Tn10 insertions in the *rfaG*, *rfaP*, and *galU* genes involved in lipopolysaccharide core biosynthesis that affect *Escherichia coli* adhesion. Arch. Microbiol. **172:**1–8.
9. **Gilman, R. H., R. B. Hornick, W. E. Woodard, H. L. DuPont, M. J. Snyder, M. M. Levine, and J. P. Libonati.** 1977. Evaluation of a UDP-glucose-4-epimeraseless mutant of *Salmonella typhi* as a liver oral vaccine. J. Infect. Dis. **136:**717–723.
10. **Gray, B. H., and J. R. Haseman.** 1994. Bactericidal activity of synthetic peptides based on the structure of the 55-kilodalton bactericidal protein from human neutrophils. Infect. Immun. **62:**2732–2739.
11. **Haldimann, A., and B. L. Wanner.** 2001. Conditional-replication, integration, excision, and retrieval plasmid-host systems for gene structure-function studies of bacteria. J. Bacteriol. **183:**6384–6393.
12. **Hohmann, A., G. Schmidt, and D. Rowley.** 1979. Intestinal and serum antibody responses in mice after oral immunization with *Salmonella*, *Escherichia coli*, and *Salmonella-Escherichia coli* hybrid strains. Infect. Immun. **25:**27–33.
13. **Little, R. G., D. N. Kelner, E. Lim, D. J. Burke, and P. J. Conlon.** 1994. Functional domains of recombinant bactericidal/permeability increasing protein (rBPI23). J. Biol. Chem. **269:**1865–1872.
14. **McCallum, K. L., G. Schoenhals, D. Laakso, B. Clarke, and C. Whitfield.** 1989. A high-molecular-weight fraction of smooth lipopolysaccharide in *Klebsiella* serotype O1:K20 contains a unique O-antigen epitope and determines resistance to nonspecific serum killing. Infect. Immun. **57:**3816–3822.
15. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
16. **Miller, V. L., and J. J. Mekalanos.** 1988. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. J. Bacteriol. **170:**2575–2583.
17. **Nesper, J., C. M. Lauriano, K. E. Klose, D. Kapfhammer, A. Kraiss, and J. Reidl.** 2001. Characterization of *Vibrio cholerae* O1 El tor *galU* and *galE* mutants: influence on lipopolysaccharide structure, colonization, and biofilm formation. Infect. Immun. **69:**435–445.
18. **Ornellas, E. P., and B. A. Stocker.** 1974. Relation of lipopolysaccharide character to P1 sensitivity in *Salmonella typhimurium*. Virology **60:**491–502.
19. **Paton, A. W., E. Voss, P. A. Manning, and J. C. Paton.** 1998. Antibodies to lipopolysaccharide block adherence of Shiga toxin-producing *Escherichia coli* to human intestinal epithelial (Henle 407) cells. Microb. Pathog. **24:**57–63.
20. **Paton, J. C., and A. W. Paton.** 1998. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev. **11:**450–479.
21. **Perna, N. T., G. Plunkett III, V. Burland, B. Mau, J. D. Glasner, D. J. Rose, G. F. Mayhew, P. S. Evans, J. Gregor, H. A. Kirkpatrick, G. Posfai, J. Hackett, S. Klink, A. Boutin, Y. Shao, L. Miller, E. J. Grotbeck, N. W. Davis, A. Lim, E. T. Dimalanta, K. D. Potamousis, J. Apodaca, T. S. Anantharaman, J. Lin, G. Yen, D. C. Schwartz, R. A. Welch, and F. R. Blattner.** 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature **409:**529–533.
22. **Perry, M. B., L. MacLean, and D. W. Griffith.** 1986. Structure of the O-chain polysaccharide of the phenol-phase soluble lipopolysaccharide of *Escherichia coli* 0:157:H7. Biochem. Cell Biol. **64:**21–28.
23. **Peschel, A.** 2002. How do bacteria resist human antimicrobial peptides? Trends Microbiol. **10:**179–186.
24. **Pluschke, G., and M. Achtman.** 1984. Degree of antibody-independent activation of the classical complement pathway by K1 *Escherichia coli* differs with O antigen type and correlates with virulence of meningitis in newborns. Infect. Immun. **43:**684–692.
25. **Raetz, C. R. H.** 1996. Bacterial lipopolysaccharides: a remarkable family of bioactive macroamphiles, p. 1035–1063. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, DC.
26. **Rajashekara, G., D. A. Glover, M. Banai, D. O'Callaghan, and G. A. Splitter.** 2006. Attenuated bioluminescent *Brucella melitensis* mutants GR019 (*virB4*), GR024 (*galE*), and GR026 (BMEI1090-BMEI1091) confer protection in mice. Infect. Immun. **74:**2925–2936.
27. **Ritchie, J. M., C. M. Thorpe, A. B. Rogers, and M. K. Waldor.** 2003. Critical

roles for *stx2*, *eae*, and *tir* in enterohemorrhagic *Escherichia coli*-induced diarrhea and intestinal inflammation in infant rabbits. Infect. Immun. **71:** 7129–7139.

28. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Mol. Biol. Rev. **53:**1–24.
29. **West, N. P., P. Sansonetti, J. Mounier, R. M. Exley, C. Parsot, S. Guadagnini, M. C. Prevost, A. Prochnicka-Chalufour, M. Delepierre, M. Tanguy, and C. M. Tang.** 2005. Optimization of virulence functions through glucosylation of Shigella LPS. Science **307:**1313–1317.

Editor: A. D. O'Brien