Spontaneous Deletion of a 209-Kilobase-Pair Fragment from the 
*Escherichia coli* Genome Occurs with Acquisition of Resistance 
to an Assortment of Infectious Phages

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Among the many systems available for heterologous protein expression, the gram-negative bacterium *Escherichia coli* remains one of the most attractive because of its ability to grow rapidly and at high density on inexpensive substrates, its well-characterized genetics, and the availability of a large number of cloning vectors (13, 19). However, phage infection of *E. coli* cultures can lead to serious problems, including a complete loss of the desired bioproduct and the spread of the destructive bacteriophages. Such problems with culture lysis may reappear suddenly and frequently within the same laboratory. Decontamination is especially difficult in a large factory. If a phage propagated in a bioreactor can spread throughout the plant, it may survive for a long period of time and cause recurrent problems. Although the deleterious effects of bacteriophages are well recognized, there are relatively few published reports addressing this problem.

Extensive work has been conducted to select or breed phage-resistant strains in the dairy industry (7). Dairy fermentation remains susceptible to phage infection, since pasteurized milk is not completely sterilized. In recent years, genetic strategies to improve the phage resistance of bacterial strains developed from knowledge about natural phage defense systems (4, 11, 23). Major categories of natural phage defenses include adsorption barriers, abortive infection mechanisms, and DNA restriction and modification systems (12). One long-term protection strategy is to select phage-resistant mutants with altered adsorption characteristics. These mutants can result from mechanisms that change carbohydrate composition or alter specific phage protein receptors. Nevertheless, in most strains isolated so far, the underlying mutations have not been well characterized. Few alterations in phage receptors have been correlated with a resistance phenotype at the molecular level (16, 17, 18, 24). Therefore, for this type of phage defense strategy to provide protection against different phage species that can use alternative receptors, it is important to understand common and essential features of the phage adsorption process.

Previously, we investigated the interaction between *E. coli* O157:H7 and its specific bacteriophage PP01 in chemostat continuous culture (15). Following PP01 phage addition, the observed *E. coli* O157:H7 cell lysis was greater than 4 orders of magnitude. However, the appearance of a series of phage-resistant *E. coli* organisms, which showed reduced efficiency of plating when PP01 phage was used, led to an increase in the cell concentration in the culture. This observation led us to the idea of strengthening an *E. coli* strain against phage attack by enrichment methods during continuous culture in a chemostat. As detailed in this report, the sequential exposures to four different phage mixtures prepared from sewage influent succeeded in producing a multiple-phage-resistant *E. coli* strain. This study describes the methodology for breeding phage resistance and the genetic nature of the resultant *E. coli* mutant.

**MATERIALS AND METHODS**

**Bacterial strains, bacteriophages, and preparation of phage mixtures.** *E. coli* JM109 was used as a model strain for breeding phage resistance. *E. coli* (ME8305) RK4784 (ompC deletion mutant of *E. coli* strain K-12 [F− Δ(argF-lac) recA1 deoC1 ΔwbbL ΔompC]) and *E. coli* (ME8307) RK4784 (ompA deletion mutant of *E. coli* strain K-12 [F− Δ(argF-lac) recA1 deoC1 ΔwbbB ΔompA]) were kindly provided by the National Institute of Genetics, Japan. In batch culture, *E. coli* was cultured overnight in 2 ml of Luria-Bertani (LB) broth at 37°C with
shaking (120 rpm). The optical density of the medium at 600 nm (OD_{600}) was measured using a KL-21 spectrophotometer (Hitachi High-Technologies Corp.) to estimate the cell concentration. Thirty-eight bacteriophages used in this study, IS01 to IS33, IP008, IP052, SP13, EP16, and ECV43, were screened from sewage influent and activated sludge sample from a wastewater treatment plant in Japan. Samples for phage mixture preparations were taken from an influent to the wastewater treatment plant. The number of inhabitants served by this plant was about 200,000. Most of the contamination was of human origin. The plant treated no effluent from animal farms or industries. The daily volume of the influent was 28,000 m^3. The representative sewage influent qualities were as follows: biological oxygen demand, 260 mg/liter; suspended solids, 190 mg/liter; and coliform bacterial count, 600,000 cells/ml. The sewage sample was centrifuged (11,100 g, 5 min). Then, the obtained supernatant (10 ml) was carefully transferred to a new sterilized tube, mixed with chloroform (100 g), and incubated for 20 min by shaking (120 rpm), centrifuged (11,100 g, 5 min at 4°C to separate the supernatant and cell pellets. The phage titer of the supernatant was determined by serial dilution with sterile SM buffer (10 mM MgSO_4, 100 mM NaCl, 0.01% gelatin, and 50 mM Tris-HCl [pH, 7.5]), followed by a plaque assay on lawns of E. coli JM109. The cell pellets were washed and resuspended in and diluted with phosphate-buffered saline, and the viability of the cells was determined by plating them onto LB agar. All assays were done in triplicate. After 150 to 200 h of chemostat continuous culture, samples of the cultures were plated onto LB agar, and a colony was used as a seed for the next run. This manipulation was repeated four times (chemostat runs A to D).

**Construction of the ompC expression plasmid.** The ompC DNA fragment was PCR amplified from chromosomal DNA of E. coli K-12 with primers +OmpC and −OmpC*. The oligonucleotide primers used for PCR are listed in Table 1. The primers contain recognition sites of the restriction enzymes NcoI and SalI, respectively. The PCR products were digested with NcoI and SalI and inserted into the NcoI/SalI-digested pTV118N (Takara, Kyoto, Japan) to produce plasmid pOmpC.

**Spot test.** Seventy strains of E. coli, screened from sewage influent from February to April in 2004 using Chromocult coliform agar medium (Merck), were used for the selection of 38 phages (IS01 to IS33, IP008, IP052, SP13, EP16, ECV43) (Table 2). E. coli was mixed with 0.5% agar and overlaid on an LB plate. Then, 2 μl of phage lysate (>10^9 PFU/ml) was dropped on that plate and incubated overnight. Each spot test was conducted once. The 38 phages were discriminated by the sampling day, place, and host range.

**Analysis of the outer membrane components of E. coli.** Outer membrane proteins were purified as previously described (16). Following denaturation with sodium dodecyl sulfate (SDS), the cell outer membrane proteins were separated on a 12% polyacrylamide gel containing SDS (SDS-12% PAGE) and stained with Coomassie brilliant blue R-250. Lipopolysaccharide (LPS) was prepared by hot phenol-water extraction (20) and by proteinase K digestion (10). LPS was separated on an SDS-15% PAGE gel and detected by using Sil-Best Stain-Neo (Nacalai Tesque, Inc., Japan). Silver staining of LPS gels enabled detection of O-antigen-free LPS, i.e., LPS containing only the R-core region and the lipid A complex.

**Cell growth and β-galactosidase assay.** E. coli cells were grown in 4 ml LB or M9G medium (6 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.2 g of MgSO_4·6 H_2O, 0.5 g of NaCl, 1 g of NH_4Cl, 10 g of Casamino Acids, and 2 g of glucose [per liter of water]) at 37°C with shaking. Cell concentrations were estimated by measuring the OD_{600} of the medium. The OD_{600} was measured using a biophotorecorder (model TV5062CA; Advantec Corp., Japan) to estimate the cell concentration. To analyze the production of a recombinant protein, the phage-resistant strain (D198) and the parent strain (JM109) were each transformed by the plasmid pUC118 and incubated in LB medium supplemented with ampicillin (50 mg/liter) and IPTG (isopropyl-β-d-thiogalactopyranoside; 10 mM). When the OD_{600} reached 0.6, the cells were separated by centrifugation, and β-galactosidase activity was assayed (14).

**RESULTS**

**Growth of E. coli JM109 and sewage phage samples in continuous culture.** Chemostat continuous culture (D, 1.0 h⁻¹)
was employed for analyzing the effect of phage mixtures on *E. coli* growth (Fig. 1). Before the addition of phage samples, *E. coli* cells were incubated overnight and achieved a cell concentration of approximately $5 \times 10^8$ CFU/ml. Next, a phage mixture prepared from sewage influent, designated sample A, was added to the flask at time zero. The phage concentration in sample A, when assayed on *E. coli* JM109, was 103.3 PFU/ml. Initially after infection with phage sample A, there was an approximately $10^3$-fold decrease in bacterial concentration that was accompanied by an approximately $10^5$-fold increase in phage concentration. However, *E. coli* was not washed out of the system. The cell concentration ascended again to a level of $10^6$ CFU/ml 10 hours after phage infection.

Three *E. coli* colonies were isolated from the spent culture medium, and the corresponding strains were designated A54, A102, and A150. The number following “A” in the designations indicates the time after phage addition (in hours) at which the bacteria were removed from the chemostat. Plaque assays were conducted by using phage sample A on lawns of *E. coli* JM109 (wild type), A54, A102, and A150 (Fig. 1, tables). The PFU values for the last three strains were all less than 30% that of the parent strain, JM109. However, plaque assays indicated that A54, A102, and A150 were not completely resistant to all phages present in sample A. Some of the phages in the mixture could have been washed out during the early period of the chemostat run, and they might remain infectious to A54, A102, and A150. Phage resistance of these *E. coli* strains was further assessed by determining growth curves during batch culturing (Fig. 2). After the addition of phage sample A to the exponentially growing *E. coli* wild type, JM109, the marked

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### Table 2. Susceptibility of phage-resistant *E. coli* strains against 40 phages

| Phage | JM109 | A54 | A102 | A150 | B54 | B102 | B150 | C54 | C150 | C292 | D54 | D102 | D150 | D198/OmpC | K-12 ΔompC |
|-------|-------|-----|------|------|-----|------|------|-----|------|------|-----|------|------|-----------|-----------|
| IS01  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS02  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●         | ○         |
| IS03  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS04  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS05  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS06  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS07  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS08  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS09  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS10  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS11  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS12  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS13  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS14  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS15  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS16  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS17  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS18  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS19  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS20  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS21  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS22  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS23  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS24  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS25  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS26  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS27  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS28  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS29  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS30  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS31  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS32  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IS33  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IP008 | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| IP052 | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| SP13  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| EP16  | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| ECV43 | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| T2    | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |
| T4    | ○     | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ●   | ○    | ○    | ○         | ○         |

* The phages are classified based on their receptors. Group A phages (IS03 and IS24) produced plaques on the lawn of D198/OmpC, and group B phages (IS01 to IS02, IS04, IS06, IS08, IS15, IS25 to IS27, IS29 to IS33) produced plaques on the lawn of K-12 ΔompC. Group C phages (IS05, IS07, IS09 to IS14, IS16 to IS20, T2, T4) produced plaques on the lawns of both D198/OmpC and K-12 ΔompC, while group D phages (IS21 to IS23, EP16, ECV043) produced plaques on the lawns of neither mutant.

* a plaque formed.

* Transformant of D198 by pOmpC.
A decrease in OD 660 was presumably due to cell lysis. However, no such decrease in the culture turbidity was observed with the phage-resistant strains, A54, A102, and A150.

Strain A150 was used to inoculate the second chemostat culture (run B), to which a different sewage-derived phage mixture, sample B, was added. The phage concentration in sample B, when assayed with E. coli strain A150, was 663 PFU/ml. Chemostat continuous culture was applied for further breeding of phage-resistant cells. As occurred during the first chemostat run, when the phage sample was added to the continuous culture, there was a sudden drop in the E. coli cell concentration and a concomitant increase in the phage concentration (assayed by infection of E. coli JM109 cells). Then, recovery of cell concentration was observed, as in the case of run A. Four phage-resistant derivatives were purified from the continuous culture (strains B54, B102, B150, and B174) and used as the host strains in the plaque assay with phage sample B. No plaques were detected with strain B102, B150, or B174.

The same procedure was applied twice more using different phage mixtures, samples C and D, in chemostat runs inoculated with strains B174 and C102, respectively (Fig. 1). A phage-resistant strain, designated D198, was finally isolated from the final run, run D. D198 produced no plaques when assayed with another phage mixture, sample E.

Susceptibility of E. coli isolates to different phages. The transition of E. coli isolates from being phage susceptible to being phage resistant in this experiment was analyzed by a phage spot test (Table 2). The phages used were isolated from sewage influent or activated sludge of a wastewater treatment plant. These phages showed a different host range among E. coli cells isolated from sewage influent (data not shown). Three strains that were isolated from run A, A54, A102, and A150 (Fig. 1), were susceptible to all of the individual phages tested. As the chemostat continuous culturing progressed, the E. coli isolates acquired resistance to an increasing number of individual phages. The mutations responsible for the phage resis-
The increase of OmpF production in the D198, an ompC deletion mutant, and an ompC deletion mutant. To summarize the above analysis, the bacteria isolated at a later time were susceptible to a phage to which an earlier isolate was resistant. For example, D54 was resistant to IS01, yet D102 was susceptible to the same phage. D198 was resistant to all phages except SP16 and T2.

The susceptibility of D198 to different phage mixtures, samples D to M, was analyzed by the relative phage titer, the ratio of the number of plaques formed on a lawn of D198 to the number formed on a lawn of its parent strain, JM109.

**Outer membrane protein analysis.** The levels of three *E. coli* major outer membrane proteins, OmpA, OmpC, and OmpF, were compared in the SDS-12% PAGE profiles of JM109, D198, an ompA deletion mutant, and an ompC deletion mutant. The increase of OmpF production in the ΔompC mutant should be a result of the deletion of mifC, which is an inhibitor of OmpF located upstream of *ompC* in *E. coli* (2). The production of OmpC was not detected in D198. The loss of OmpC in D198 may account for the reduced affinity of this strain for all phages, since OmpC may serve as a receptor protein for some of these phages. To test this hypothesis, the plasmid pOmpC, which encodes OmpC of *E. coli* K-12, was constructed and introduced into D198. This transformant (D198/OmpC) was grown in the presence of IPTG to induce OmpC, and SDS-12% PAGE revealed a protein band corresponding in size to OmpC (Fig. 4A). The susceptibility of D198 to the sewage phages was partly restored by this complementation, and 17 phages regained infectivity to strain D198/OmpC (Table 2). However, OmpC complementation did not completely restore susceptibility. The specific deletion of *ompC* from *E. coli* K-12 led to the resistance to seven phages, IS03, IS21 to IS24, EP16, and ECV43 (Table 2).

LPS was found in both the large-molecular-size (>30-kDa) and small-molecular-size (<20-kDa) ranges in both JM109 and D198. However, the pattern of small-molecular-size LPS was altered (Fig. 4B).

**Identification of the D198 genetic deletion.** Since D198 did not produce OmpC, genetic analysis of the *ompC* region was conducted by PCR. The relative locations of the primers used for PCR are summarized in Fig. 5A. The primer set +ompC/-OmpC did not generate an amplicon with template DNA from D198, suggesting a deletion (data not shown). However, analysis of the region upstream of *ompC* by PCR with various primer sets (+U6/-U4, +U5/-U3, and +U4/-U2) revealed fragments of the expected sizes with template DNA from either JM109 or D198, indicating that the genome of D198 was unaltered in this region (data not shown).

In contrast, the genomic region downstream of and encompassing *ompC* appeared to be missing in D198, since PCRs with many primer sets (including primer sets +U3/-OmpC, +U3/-U1, and +OmpC/-D1) generated DNA fragments of the expected sizes with template DNA from JM109 but not D198 (data not shown). To define the endpoints of the deletion, the upstream primer +U3 was used with a series of downstream primers (D1 to D5). DNA of the expected size was observed with the JM109 template, whereas only the primer set +U3/-D2 produced a distinct band with the D198 template (Fig. 5B). However, the size of the fragment was consistent with a significant chromosomal deletion in D198. The +U2/-D2 and +U1/-D2 primer sets also generated amplicons with D198 template DNA (Fig. 5B). The PCR-generated fragment produced by primer pair +U2/-D2 was digested with BamHI and HindIII and ligated into a similarly digested pUC118 vector. The DNA sequence of the cloned fragment revealed a segment corresponding to the primer +U1 and its 7-bp downstream region. This segment was fused with DNA corresponding exactly to the sequence of a region of the *E. coli* K-12 genome (NCBI/GenBank accession no. NC-000913) that is normally 207.6 kbp downstream from *ompC*. Inadvertently, part of primer −D2 (5'-CCCAAGCTTATCCCAGGCATCCGCTTC-3') carries a sequence complementary to this region (shown underlined). To summarize the above analysis, phage-resistant *E. coli* JM109 (i.e., D198) lost a genomic fragment of approximately 209.4 kbp from *ompC* to *wbbL* that encodes the rhamnosyl transferase involved in LPS biosynthesis.
Recombinant protein production. To determine whether the genetic changes affecting phage resistance would impact the potential use of D198 as a host for recombinant plasmids, the growth rates of this strain were compared to those of the parent strain, JM109. As shown in Fig. 6, the growth rates of both strains were indistinguishable on rich medium or minimal medium. To compare the expression levels of a recombinant protein, these two strains were transformed with a pUC118 plasmid carrying lacZ. D198 and JM109 produced comparable levels of β-galactosidase (LacZ) enzyme activity (Fig. 6).

DISCUSSION

The most likely source of phage contamination in the E. coli culturing process is human, since E. coli is one of the main inhabitants of the gastrointestinal tracts of warm-blooded animals. Previously, we reported that a relatively abundant coliphage, with concentrations of 1,000 to 10,000 PFU/ml determined by using three different E. coli strains, was detected in sewage influent (21). In our current study, the sequential exposure of E. coli to multiple-phage mixtures from sewage was used to develop JM109 derivatives that are resistant to many different phages. This approach builds on previous demonstrations that long phage exposure of E. coli selects for resistant cells (15, 16, 22).

Susceptibility tests suggest that the mutations responsible for multiple-phage resistance accumulate sequentially (Table 2). The chemostat provides an important device for studying bacterium-phage interactions. In continuous culture, bacteria rapidly evolve resistance to phage infection. Different mutations can produce distinct resistance phenotypes that, for example, determine whether resistance is partial or complete, determine the magnitude of the physiological cost associated with resistance, and determine whether the mutation can be countered by host-range mutation in the phage. These differences determine the ability of a mutant to invade, the effect its invasion has on the population dynamics of susceptible bacteria and of the phage, and the resulting structure of the community (5). As described here, a single colony was picked up randomly and used for the isolation of phage-resistant cells at each sampling time. However, the medium in the chemostat continuous culture contains a variety of phage-resistant cells. Some of the phage-resistant isolates selected in this study could be washed out from the system in long-term studies. On the other hand, resistant cells that were not selected might grow and become dominant in the system later on.
A phage-resistant strain, D198, was obtained after exposure to four different phage mixtures. However, additional sewage samples contained phages infectious to D198 (samples F to J). Although D198 remained susceptible to some phages, the low relative phage titer suggests that the use of D198 might minimize bacteriophage attack. Furthermore, additional exposure of D198 to different phage mixtures might yield increased resistance. The methodology presented here using successive exposures to heterogeneous mixtures of phages might be applicable to the isolation of other types of bacteria, in addition to E. coli, that are resistant to multiple phages.

Phage infection starts with the adsorption of phages on the bacterial cell surface, and the host range is controlled by the interactions of the phage and its receptor. Generally, phages use outer membrane proteins and/or LPS as their receptor. PP01-resistant cells lost ompC expression due to the deletion of a 14-kbp region upstream of ompC. Outer membrane analysis of D198 also indicated the loss of OmpC production. Genotypic analysis of D198 revealed a 209.4-kbp deletion that encompasses the whole ompC and genes necessary for LPS production. The deleted genes include wbbJ (O acetyltransferase), wbbK (glucosyltransferase), and wbbL (rhamnosyl transferase) involved in LPS synthesis. LPS consists of lipid A, core oligosaccharide, and O antigen. The biogenesis of LPS is a complex multistep process. The core oligosaccharide is assembled on preformed lipid A by the sequential glycosyl transfer of each monosaccharide. In addition to the ompC deletion, deletions of transferase genes may contribute to the phage-resistant phenotype.

Since OmpC complementation did not completely restore phage susceptibility to D198, additional factors affect the phage resistance properties of this strain. Genes that are included in the 209.4-kbp deletion (besides ompC) and/or other genomic mutations in D198 appear to be involved in phage interactions. Forty phages used in this study could be classified into four groups based on the OmpC complementation test with D198 and the phenotype of an ompC deletion mutant, K-12 ΔompC (Table 2). Phages classified into group A appear to use OmpC as a receptor, since resistance results from the specific deletion of its gene in K-12 ΔompC and since susceptibility was restored in D198/ompC. Phages classified into group B do not depend on OmpC as a receptor, since its absence alone does not cause resistance. Phages in this group might use a receptor that is not produced (or not functional) in D198 because of the deletion or other unknown mutations. For phages in group C, the complementation of D198 indicates that OmpC can be used as a receptor. However, since the ompC deletion alone does not confer resistance, members of this group may also be able to use another receptor that is not made or not functional in D198. For phages in group D, it appears that OmpC is necessary but not sufficient for infection.

It is known that phage T4 uses the LPS of the outer cell envelope membrane as a receptor (8, 9). The alteration of LPS shown in Fig. 4 and the fact that genes responsible for the production of LPS were borne in the lost DNA fragment suggest that LPS plays an important role in determining the D198 phenotype. However, a complete genetic analysis of D198 has not yet been conducted.

Although the D198 genome is 4.5% smaller than that of JM109, the two strains have comparable growth rates and produce the same levels of recombinant LacZ activity (Fig. 6). As a recombinant host, E. coli is exposed to a limited and controlled set of conditions. Therefore, the genes required for survival in the gut may not be the same ones required for optimum recombinant protein production. Consistent with this theory, improvements in E. coli as a recombinant host have often involved genetic deletions (1, 3, 6). Here, we demonstrate that a large deletion surrounding ompC strengthens phage resistance, and this study provides a suitable foundation for further genomic reduction in E. coli hosts to be used for recombinant protein production.

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FIG. 6. Cell growth and β-galactosidase assay. (A) β-Galactosidase activity of E. coli cells (JM109 and D198 transformed by pUC118). 1-bars show standard deviations (n = 3). (B) Profiles of E. coli cell growth. JM109 in LB medium (open circles), D198 in LB medium (filled circles), JM109 in M9G medium (open triangles), and D198 in M9G medium (filled triangles) were used.
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