論文 / 著書情報
Article / Book Information

Title	Amino acid alterations on Gp38 of host range mutant of PP01 and evidence for OmpC null mutation in its host cell, <I>Escherichia coli</I> O157:H7
Author	Masatomo Morita, Curt R. Fischer, Katsunori Mizoguchi, Masatoshi Yoichi, Masahito Oda, Yasunori Tanji, Hajime Unno
Journal/Book name	FEMS Microbiol. Lett., Vol. 216, No. , pp. 243-248
Issue date	2002,
URL	http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1574-6968
DOI	http://dx.doi.org/10.1016/S0378-1097(02)01025-X
Note	このファイルは著者（最終）版です。 This file is author (final) version.

Title: Amino acid alterations on Gp38 of host range mutant of PP01 and evidence for OmpC null mutation in its host cell, *Escherichia coli* O157:H7

Authors: Masatomo Morita, Curt R. Fischer, Katsunori Mizoguchi, Masatoshi Yoichi, Masahito Oda, Yasunori Tanji * and Hajime Unno

Affiliations: Department of Bioengineering, Tokyo Institute of Technology

4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, *Japan*

Key words: *Escherichia coli* O157:H7, virulent bacteriophage, resistant cell, host range mutant

Corresponding information: * Corresponding author

Department of Bioengineering, Tokyo Institute of Technology

4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, *Japan*

Tel: +81-45-924-5763. Fax: +81-45-924-5818.

e-mail: ytanji@bio.titech.ac.jp

Abstract

The previously isolated T-even type coliphage PP01, which specifically infects to *Escherichia coli* O157:H7, uses the outer membrane protein OmpC as a receptor. The PP01-resistant cells had lost *ompC* expression due to the deletion of a 14 kbp region upstream of *ompC*. Two host range mutants, infective to the OmpC null mutant, were isolated and gene *38*, which codes for the receptor recognition protein Gp38, was sequenced from both host range mutant. According to the deduced amino acid sequence, the mutational alterations were found in Gp38. The most common mutations changed glutamine at position 161 and glycine at position 208 into basic amino acids.

1. Introduction

Enterohemorrhagic *Escherichia coli* serogroup O157:H7 is known to cause bloody diarrhea and hemolytic uremic syndrome in humans. It has been suggested that most *E. coli* O157:H7 infections in humans are foodborne illnesses and that domestic animals are reservoirs of *E. coli* O157:H7 [1, 2]. Phages specific for *E. coli* O157:H7 have been detected in feces from animals at relatively abundant levels [3-5]. In general, phages can be categorized into two groups: temperate phage and virulent phage, based on their life cycle. Temperate phage can mediate horizontal transfer of genes such as *stx* [6]. On the other hand, virulent phages may play a role in controlling the host cell population in gastrointestinal or other environments. Phage-mediated control of pathogenic organisms may potentially be exploited therapeutically. Several investigations have had success in animal models [7-11].

A virulent phage, previously isolated from swine stool samples, was found to infect *E. coli* O157:H7 strains with high specificity. Phage infection is initiated by the specific adsorption on the bacterial cell surface. The distal long tail fibers of T-even phages are responsible for recognizing specific cell surface receptors. In phages of the T2 family, the gene *38* product (Gp38), which is present at the tip of long tail fiber, is the determinant of the host range [12]. Analysis of deduced amino acid alignments of the tail fiber proteins revealed the PP01 was related to T2. Moreover, the specific recognition of the *E. coli* O157:H7 OmpC protein by Gp38 determines its host range [13]. However, after long coincubation of cell and phage, PP01-resistant cells appeared spontaneously. Analysis revealed that an OmpC null mutation endowed the *E. coli* O157:H7 cells with resistance against PP01 infection. On the other hand, host range

mutants of PP01, which were infective to the OmpC null mutant, were also isolated. According to analyses of host range mutants in the T2 family, mutations in gene *38* are usually either insertions caused by small duplications or amino acid alterations caused by DNA base-pair alterations. These mutations alter the receptor for the phage [14-17].

Understanding the mechanisms of mutations controlling the infectivity of phages may facilitate the design of successful phage-based therapies. In this study, we elucidated the cause of the loss of *ompC* expression in mutant *E. coli* O157:H7 cells and found that mutational alterations on Gp38 endow the PP01 phage mutants with a broad host range.

2. Materials and Methods

2.1. Bacterial strains and bacteriophages

E. coli O157:H7 ATCC43888, which does not produce either Stx1 or Stx2 because of a lack of the genes for these toxins, was used for the propagation of phages and the estimations of phage titer. The PP01-resistant strain, R01s was obtained from ATCC43888, as described previously [13]. This strain lost the production of outer membrane protein, OmpC. The strain RK4784 (*ompC* mutant of *E.coli* K-12) harboring the plasmid pOMPC1, which encodes *ompC* of *E. coli* O157:H7 ATCC43888, was also obtained in previous work [13]. In addition to them, *E. coli* K-12 W3110 [18] was used for the determination of phage susceptibility. All strains were grown in Luria-Bertani (LB) broth at 37°C. For RK4784/pOMPC1 [13], LB broth was supplemented with 5

μg/ml ampicillin and 1 mM isopropyl-β-D-thiogaractopyranoside.

The bacteriophage PP01 and its host range mutants, M01f and M01g, were from the laboratory stock. The host range mutants, M01f and M01g were obtained independently by plating PP01 onto R01s.

2.2. Southern blot analyses

Preparation of digoxigenin (DIG)-labeled probes was performed with the PCR DIG Probe Synthesis Kit (Roche Diagnostic) according to the manufacturer's instructions. Probe DIG-ompC, specific for the *ompC* of *E. coli* O157:H7 ATCC43888, was generated with primers (5'-CATGCCATGGGCATGAAAGTTAAAGTACTGTCCC-3' and 5'-CCCAAGCTTTGATTATCCTCATGCGAACG-3'), with genomic DNA of *E. coli* O157:H7 ATCC43888 as a template. In the same manner except for the primers, probe DIG-up, specific for the 1-kbp-region upstream of *ompC*, was prepared. The primers for this reaction were 5'-CGGGATCCGCGCCCCACAATGTGTCC-3' and 5'-CCCAAGCTTGGGACAGTACTTTAACTTTCAT-3'.

Genomic DNA was digested with restriction enzymes and analyzed by electrophoresis on a 1.5% agarose gel. The agarose gel containing DNA was soaked in the denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 15 min, followed by washing in the neutralization solution (1.0 M Tris, 1.5 M NaCl: pH = 8.0) for 15 min. DNA was then transferred to a nylon membrane (Millipore) by capillary action with 20×SSC (3 M NaCl, 0.3 M sodium citrate: pH = 7.0) and was fixed to the membrane by UV cross-linking at 1200 J/cm^2. For prehybridization, membranes were treated with DIG

Easy Hyb (Roche Diagnostic) and incubated at 55°C for 30min, followed by hybridization at 68°C overnight with DIG labeled probe DNA. Membranes were washed in 2×SSC with 0.1% (wt/vol) sodium dodecyl sulfate at room temprature (2 times, 5 min), followed by two washes for 15 min each at 68°C with 0.1×SSC with 0.1% (wt/vol) sodium dodecyl sulfate. Thereafter, detection of hybridized probe was conducted with the DIG Luminescent Detection Kit (Roche Diagnostic) according to the manufacturer's instructions.

2.3. Cloning and sequencing

The *Kpn*I/*Pst*I fragment of the R01s genome, hybridized with DIG-ompC, was extracted from agarose gel by GenElute Agarose Spin Columns (Sigma). This fragment was then replaced with the *Kpn*I/*Pst*I fragments of the multiple cloning site of pUC118.

PCR was used to clone the tail fiber gene *38* of host range mutants. DNA was extracted from bacteriophages with phenol-chloroform-isoamyl alcohol (25:24:1), and the DNA was then recovered by precipitation with ethanol. The precipitated DNA was dried and redissolved in distilled water; the DNA solution was then used for the PCR amplification. The upper primer was 5'-CGGGATCCGCTTCGGCCCCTTGGAG-3', and the lower primer was 5'-CCCAAGCTTGCTGCCATAGACCCTCC-3'. Underlined nucleotides indicate sequences of *Bam*HI and *Hin*dIII, respectively. PCR fragments were cloned into the *Bam*HI/*Hin*dIII site of pUC118. The resultant plasmids were designated as pMF38 and pMG38, each harboring the gene *38* of M01f or M01g, respectively.

Nucleotide sequences of the cloned fragments were determined using fluorescein

isothiocyanate-labeled universal forward (5'-CGCCAGGGTTTTCCCAGTCACGAC-3') and reverse (5'-GAGCGGATAACAATTTCACACAGG-3') primers (Shimadzu). Automated cycle sequencing was performed on a DSQ-2000L sequencer (Shimadzu) with a pretreated sample using a Thermo Sequenase Fluorescent-Labeled Primer Cycle Sequencing Kit with 7-deaza-dGTP (Amersham Pharmacia Biotech).

2.4. Construction of chimeric phages

PP01 infection against *E. coli* O157:H7 ATCC43888 carrying the plasmid pMF38 of pMG38 was conducted, followed by overnight incubation. The overnight culture was centrifuged (12000 × *g* for 5 min at 4°C) and phages infective to the R01s strain were isolated by plaque assay. A single plaque was isolated and the phage was resuspended in LB broth. The resuspension was centrifuged, filtered, and subjected again to single plaque isolation on the R01s strain. Phages that formed plaque on R01s seemingly acquired the gene *38* of host range mutant by homologous recombination. The gene *38* of the chimeric phage was confirmed to be identical to that of host range mutant. Chimeric phages were designated as PP01mf38 and PP01mg38, each harboring gene *38* of M01f and M01g, respectively.

2.5. Bacteriophage adsorption assay

R01s cells were grown in LB broth overnight. The cells were diluted to 1×10^8 colony-forming units ml^{-1} with LB broth and inoculated in 10 ml at 37°C with shaking

(120 rpm). Phage infection with the final concentration of 1×10^5 plaque-forming units ml^{-1} was performed after 60 min of incubation. After the infection, samples of 1 ml were removed periodically and centrifuged (15000 × *g* for 1 min at 4°C). The phage titer of the supernatant was determined by the plaque assay with *E. coli* O157:H7 ATCC43888. The phage titer at time 0 was defined as 100%.

3. Results

3.1. Evidence for the loss of OmpC production in R01s strian

In our previous work, analysis of the outer membrane proteins revealed that R01s had lost the outer membrane protein OmpC but increased the production of OmpF. In addition, PCR amplification of *ompC* using its genomic DNA failed. It is known that *micF*, which is inhibitor of OmpF, is located upstream of *ompC* in *E. coli* strains. The increase of OmpF production might result from the deletion of *micF*. Thus we hypothesized that the R01s deletes a region partially or completely containing *ompC* as well as an upstream region.

Initially, the deletion of genes upstream *ompC* was elucidated using the probe DIG-up corresponding to the 1-kbp-region upstream of *ompC*. Chromosomal DNA from *E. coli* O157:H7 ATCC43888 gave strong hybridization signals in a Southern blot; however, no hybridization signal was obtained for R01s. On the other hand, probe DIG-ompC, specific for the *ompC* of *E. coli* O157:H7, hybridized with both of them (data not shown). Additionally, the *Kpn*I/*Pst*I-digested R01s genome generated a ca. 700

bp fragment hybridized with DIG-ompC, which was not observed in the ATCC43888 genome digested with same restriction enzymes. Then, this 700 bp fragment was cloned and its DNA sequence was determined. This revealed that the insert, except for the first 261 bp, was identical to an internal region of *ompC* (204 - 641 bp of 1103 bp as *ompC*); however, the first 261 bp had no relation with *ompC*. According to a FASTA search, this sequence showed 100 % identity over 260 bp to a region (913-1172 bp out of 4605 total bp) of a putative membrane protein gene found at ca. 14 kbp upstream from *ompC* in *E. coli* O157:H7 strains (Fig.1). These results indicate that the 14 kbp deletion, including the first 203 bp of *ompC*, caused the loss of the OmpC production in the R01s strain.

3.2. Expansion of receptor recognition by amino acid alterations on Gp38

The host range of a phage relies on the ability of the phage to recognize different receptors. In PP01, Gp38 bound to the tip of the distal tail fibers may act as in recognition of the cellular receptor OmpC [13]. Thus the host range mutants of PP01, which were infective not only to ATCC43888 but also to the R01s strain (defective in OmpC production), may have mutations on Gp38. Two host range mutants, M01f and M01g, were isolated from independent culture and the sequence of their gene *38* was determined (Table 1). M01f mutant had three mutations on gene *38*, which were identified as Gln161Arg, Trp189Arg and Gly208Arg. Interestingly, all the mutated amino acids were changed to arginine, which is a basic amino acid and is positively charged at neutral pH. The two mutations on M01g were identified as Gln161His and Gly208Arg. Note that histidine is also basic amino acid. The two amino acid mutations in M01g-Gp38 occurred at the same positions as two of three mutations in M01f-Gp38;

however, the base-pair alterations in their DNA were different. These findings imply that switching amino acid residues Gln161 and/or Gly208 into basic amino acids might play an important role in host range expansion.

To confirm that only the mutations in gene *38* broadened the host range, a fragment of gene *38* from each mutant was cloned into a plasmid. Then, *E. coli* ATCC43888 cells carrying these plasmids were infected with PP01. Chimeric phages were found at frequencies typical of homologous recombination, which is well above those of spontaneous mutation [20, 21]. Therefore, the mutations in gene *38* seemed to be the determinant for the broad host range. Instead of M01f and M01g, chimeric phages, PP01mf38 and PP01mg38, were further characterized to clarify the effect of mutations in gene *38*.

The host ranges of PP01mf38 and PP01mg38 were examined by the spot test assay, which demonstrated that they were infective to R01s. Both of them were also infective to RK4784/pOMPC1; however, they did not form plaques on *E. coli* K-12 W3110 strain (Table 2). The wild-type PP01 utilized only OmpC derived from O157:H7 and not that of K-12 as its receptor molecule. Similarly, chimeric phages did not lost affinity for OmpC of O157:H7 and gained the ability to use other unidentified cellular receptor(s).

To examine the affinity of each phage to the R01s strain, the phage adsorption assay was conducted (Fig. 2). PP01 did not bind to R01s cells, whereas adsorption of chimeric phages was manifest as the decrease of phage titer. Comparing their affinity, adsorption of PP01mg38 was lower than that of PP01mf38. Gp38 of PP01mf38 was more positively charged due to the three amino acid alterations into arginine than that of PP01mg38. Generally, the cell surface of gram-negative bacteria is covered with negatively charged outer membrane elements, e.g. lipopolysaccharide (LPS) [22].

Therefore, the electric interaction between Gp38 and bacterial cell surface might assist phage to access the bacteria.

4. Discussion

The general architecture of Gp38 of T2 family is the same. The two conserved regions encompass about 120 amino terminal and 25 carboxyl terminal residues, respectively. The area between these is variable and is interrupted by conserved glycine-rich regions [21, 23]. Alterations causing receptor switches have mostly occurred in the variable regions, which are proposed to be the receptor-recognizing domains of Gp38 [14-17]. The Gp38 of the PP01 host range mutant also has amino acids alterations within the variable regions. This result supports that these regions are responsible for specific attachment with the *E. coli* O157:H7 OmpC protein. However, host range mutants of PP01 did not lose the ability to use the OmpC of *E. coli* O157:H7 as a receptor. They can recognize other elements peculiar to the *E. coli* O157:H7 cell surface in addition to OmpC. In the lipopolysaccharide of *E. coli,* there are five distinct core oligosaccharide structures, designated K-12 and R1 to R4 [24]. *E. coli* O157:H7 has the R3 type, which is different from *E. coli* K-12. A host range mutant of the T-even type coliphage Ox2, whose receptor is the outer membrane protein OmpA as a receptor, gained the ability to recognize a LPS because of the mutational alterations in Gp38 [16]. Similarly, host range mutants of PP01 may utilize both OmpC protein and the core oligosaccharide of *E. coli* O157:H7 LPS as a receptor.

The PP01-resistant strain R01s was originally isolated from the culture of *E. coli*

O157:H7 ATCC43888 and PP01 phage, without mutagen [13]. The R01s cell lost the 14 kbp genome upstream from *ompC* instead gained resistance to PP01 phage. The mechanism of gene deletion is not clear; however, this mutation is advantageous for cell growth in presence of the PP01 phage. On the other hand, PP01 also mutated so as to be infective to OmpC defective *E. coli* O157:H7. Predator-prey relationships between them may bring about coevolution, as the result of genetic mutations. Both the phage and bacteria seemed to be continuously evolving in a mutual, ever-escalating "arms race". This work is the first to track genetic pathways that this arms race can take.

Acknowledgements

We thank F. Arisaka (Tokyo Institute of Technology, Japan) and M. Wachi (Tokyo Institute of Technology, Japan) for helpful discussions. This research was financially supported in part by a grant (13450340) of the Japanese Ministry of Education, Science, Sports and Culture.

References

[1] Hancock, D. D., Besser, T. E. and Rice, D. H. (1998) Ecology of *Escherichia coli* O157:H7 in cattle and impact of management practices. In: *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains (Kaper, J. B. and O'Brien, A. D., Eds.), pp. 85-91. American Society for Microbiology, Washington, DC.

[2] Vuddhakul, V., Patararunggrong, N., Pungrasamee, P., Jitsurong, S., Morigaki, T., Asai, N. and Nishibuchi, M. (2000) Isolation and characterization of *Escherichia coli* O157 from retail beef and bovine feces in Thailand. FEMS Microbiol. Lett. 182, 343-347.

[3] Ronner, A.B. and Cliver, D.O. (1990) Isolation of characterization of coliphage specific for *Escherichia coli* O157:H7. J. Food Prot. 53, 944-947.

[4] Goodridge, L., Chen, J. and Griffiths, M. (1999) Development and characterization of a fluorescent-bacteriophage assay for detection of *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 65, 1397-1404.

[5] Kudva, I. T., Jelacic, S., Tarr, P. I., Youderian, P. and Hovde., C. J. (1999) Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophage. Appl. Environ. Microbiol. 65, 3767-3773.

[6] O'Brien, A. D., Newland, J. W., Miller, S. F., Holmes, R. K., Smith, H. W. and Formal, S. B. (1984) Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science 226, 694-696.

[7] Smith, H. W. and Huggins, M, B. (1982) Successful treatment of experimental *Escherichia coli* infections in mice using phage: its general superiority over antibiotics. J. Gen. Microbiol. 128, 307-318.

[8] Merril, C. R., Biswas, B., Carton, R., Jensen, N. C., Creed, G. J., Zullo, S. and Adhya, S. (1996) Long-circulating bacteriophage as antibacterial agents. Proc. Natl. Acad. Sci. USA. 93, 3188-3192.

[9] Barrow, P., Lovell, M. and Berchieri, Jr. A. (1998) Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. Clin. Diagn. Lab. Immunol. 5, 294-298.

[10] Park, S. C., Shimamura, I., Fukunaga, M., Mori, K. and Nakai, T. (2000) Isolation of bacteriophage specific to a fish pathogen, *Pseudomonas plecoglossicida*, as a candidate for disease control. Appl. Environ. Microbiol. 66, 1416-1422.

[11] Biswas, B., Adhya, S., Washart, P., Paul, B., Trostel, A. N., Powell, B., Carton, R. and Merril, C. R. (2002) Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. Infect. Immun. 70, 204-210.

[12] Riede, I., Drexler, K., Schwarz, H. and Henning, U. (1987) T-even-type bacteriophage use an adhesin for recognition of cellular receptors. J. Mol. Biol. 194, 23-30.

[13] Morita, M., Tanji, Y., Mizoguchi, K., Akitsu, T., Kijima, N. and Unno, H. (2002) Characterization of a virulent bacteriophage specific for *Escherichia coli* O157:H7 and analysis of its cellular receptor and two tail fiber genes. FEMS Microbiol. Lett. 211, 77-83.

[14] Riede, I., Drexler, K., Eschbach, M-L. and Henning, U. (1987) DNA sequence of genes *38* encoding a receptor-recognizing protein of bacteriophages T2, K3 and of K3 host range mutants. J. Mol. Biol. 194, 31-30.

[15] Drexler, K., Riede, I., Montag, D., Eschbach, M-L. and Henning, U. (1989) Receptor specificity of the *Escherichia coli* T-even type phage Ox2. Mutataional alternations in host range mutants. J. Mol. Biol. 207, 797-803.

[16] Drexler, K., Dannull, J., Hindennach, I., Mutschler, B. and Henning, U. (1991) Single mutations in a gene for a tail fiber component of an *Escherichia coli* phage can cause an extension from a protein to a carbohydrate as a receptor. J. Mol. Biol. 219, 655-663.

[17] Hashemolhosseini, S., Holmes, Z., Mutschler, B. and Henning, U. (1994) Alterations of receptor specificities of coliphages of the T2 family. J. Mol. Biol. 240, 105-110.

[18] Jensen, K.F. (1993) The *Escherichia coli* K-12 “wild types” W3110 and MG1655 have an *rph* frameshift mutation that leads to pyrimidine starvation due to low *pyrE* expression levels. J. Bacteriol. 175, 3401-3407.

[19] Perna, N.T., Plunket III, G., Burland, V., Mau, B., Glasner, J.D., Rose, D.J., Mayhew, G.F., Evans, P.S., Gregor, J., Kirkpatrick, H.A., Pósfai, G., Hackett, J., Klink, S., Boutin, A., Shao, Y., Miller, L., Grotbec, E.J., Davis, N.W., Lim, A., Dimalanta, E.T., Potamousis, K.D., Apodaca, J., Anantharaman, T.S., Lin, J., Yen, G., Schwartz, D.C., Welch, R.A. and Blattner, F.R. (2001) Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature 409, 529-533.

[20] Mattson, T., Houwe, G. V., Bolle, A., Selzer, G. and Epstein, R. (1977) Genetic identification of cloned fragments of bacteriophage T4 DNA and complementation by some clones containing early T4 genes. Mol. Gen. Genet. 154, 319-326.

[21] Tétart, F., Desplats, C. and Krisch, H.M. (1998) Genome plasticity in the distal tail fiber locus of the T-even bacteriophage: recombination between conserved motifs swaps adhesin specificity. J. Mol. Biol. 282, 543-556.

[22] Nikaido, H. and Vaara, M. (1985) Molecular basis of bacterial outer membrane permeability. Micobiol. Rev. 49, 1-32.

[23] Montag, D., Riede, I., Eschbach, M.-L., Degen, M. and Henning, U. (1987) Receptor-recognizing proteins of T-even type bacteriophages. Constant and hypervariable regions and an unusual case of evolution. J. Mol. Biol. 196, 165-174.

[24] Amor, K., Heinrichs, D. E., Frirdich, E., Ziebell, K., Johnson, R. P. and Whitfield,

C. (2000) Distribution of core oligosaccharide types in lipopolysaccharides from *Escherichia coli*. Infect. Immun. 68, 1116-1124.

Figure Legend

Fig. 1. DNA sequence of the interrupted region of R01s genome. The sequence in bold is identical to *ompC* of O157:H7, and the sequence in italic is identical to the region 14 kbp upstream from *ompC*. The lower case "t" is not common to either of them. Arrows indicate putative open reading frames based on *E. coli* O157:H7 EDL933 genome [14]. Bar indicates size of 1 kbp.

Fig. 2. Phage adsorption on the strain R01s. Relative phage titer in the supernatant of a mixture containing each phage and R01s was determined. Phage concentration at the time 0 was ca. 10^5 PFU ml^{-1}. Symbols: PP01 (circle). PP01mf38 (square). PP01mg38 (triangle).

Table 1
Mutations on gene *38* of the host range mutants

Phage	codon at 161 aa (aa alteration)	codon at 189 aa (aa alteration)	codon at 208 aa (aa alteration)
PP01	CAA	TGG	GGG
M01f	CGA (Gln161Arg)	CGG (Trp189Arg)	AGG (Gly208Arg)
M01g	CAC (Gln161His)	TGG	CGG (Gly208Arg)

Table 2
Infectivity of PP01 and chimeric phages with mutant tail fiber gene *38* [a]

	PP01	PP01mf38	PP01mg38
ATCC43888	+	+	+
R01s	−	+	+
W3110	−	−	−
RK4784/pOMPC1	+	+	+

[a] Plaque formation was confirmed by spot test assay. +, −: presence or absence of plaque formation.