The Journal of Microbiology, September 1999, p. 168-174
Vol. 37, No. 3

Cloning and Nucleotide Sequence Analysis of Verotoxin Gene from *Escherichia coli* O157 KNIH317 Isolated in Korea

Yong-Chjun Park, Hee-Jung Shin, and Young-Chang Kim*

School of Life Sciences, Chungbuk National University, Cheongju 361-763, Korea

(Received May 19, 1999 / Accepted June 28, 1999)

***Escherichia coli* O157 is an important pathogenic organism which causes diarrhea, haemorrhagic colitis, and haemolytic ureamic syndrome (HUS) in human. *E. coli* O157 KNIH317 was isolated from patients suffering with HUS in Korea. We designed a primer set for cloning shiga-like toxin (*slt*) gene. The amplified PCR product was used to Southern and colony hybridization as a probe. As a result, we cloned 4.5-kb *Kpn*I fragment containing the *slt* gene encoding shiga-like toxin from chromosomal DNA of *E. coli* O157 KNIH317. This recombinant plasmid was named pOVT45. *E. coli* XL1-Blue harboring pOVT45 showed cytotoxicity in Vero cells. We sequenced the *slt* gene of this strain. The A- subunit gene of the *slt* was composed of 960 base pairs with ATG initiation codon and TAA termination codon. The B-subunit was composed of 270 base pairs with ATG initiation codon and TGA termination codon. Nucleotide sequence comparison of the *slt* gene exhibited 100%, 98.4%, 93.7%, and 93.7% identity with that of shiga-like toxin type II (*slt*II) of *E. coli* bacteriophage 933W. variant *slt* of *E. coli*, *slt* of *E. coli*, and variant *slt*II of *E. coli*, respectively. From these results, it was concluded that the cloned *slt* gene belongs to SltII family and that the strain used in this study may be a lysogeny of *E. coli* bacteriophage 933W.**

Key words: Cloning, *Escherichia coli* O157 KNIH317, sequence, *slt*II gene, verotoxin

Certain strains of *E. coli*, associated with diarrhea, haemorrhagic colitis, and haemolytic ureamic syndrome (HUS), have been shown to produce cytotoxins (referred to shiga-like toxin) which are very similar to shiga toxin (Sht) produced by *Shigella dysenteriae* type I (15). These toxins are called as shiga-like toxin or verotoxin due to their cytotoxic activity in Vero cells. The major types (SltI and SltII or VTI and VTII) of shiga-toxins have been distinguished by serological method as well as nucleotide sequence analysis. SltI (or VTI) can be neutralized by antiserum prepared against purified shiga toxin, and its DNA sequence differs by only 4 bp in the A-subunit gene (resulting in a single amino Nid change). The B-subunit genes of SltI and Sht are identical (11, 24). However, SltII (or VTII) is not neutralized by antiserum of shiga toxin, and there is only about 60% homology between the nt and aa sequences of A-and B-subunit genes of SltI and SltII (23). As a variant of SltII, SltIIv (or VTIIv) is produced by *E. coli* strains associated with edema disease and is neutralized by antiserum to *Slt*II. *Slt*II and SltIIv can cross-react immunologically with each other and share 91% homology in nucleotide sequence. Sht, SltI, and SltII are compound toxins, consisting of a single enzymatically active A-subunit, which inhibits eukaryotic protein synthesis. The pentameric B-subunit is responsible for binding to glycolipid receptors in target cell membranes (10).

Unlike *sht* gene, which is chromosomally encoded, *slt*I and *slt*II genes are generally encoded on lambdoid bacteriophages (17). However, variant *slt*II genes have been isolated from the chromosomal DNA of *E. coli* strains from both human and animal sources (6).

Among these pathogens, Enterohemorrhagic *E. coli* (EHEC) O157 is highly pathogenic to human and has been isolated from patients throughout the world. EHEC O157 can be transmitted through contaminated food and water, as well as via infected persons. Clinical microbiologists are increasingly asked not only to identify, but also to type O157 to clarify the chain of infection. The typing methods currently employed include phage typing (1), multi-locus enzyme electrophoretic typing (27), *slt* genotyping (7), plasmid typing (13), random amplified polymorphic DNA fingerprinting (28), and genomic DNA restriction fragment length polymorphism (RFLP) analysis (3, 18). Molecular methods have demonstrated that O157 isolates have potential to rapidly change their genotypic

* To whom correspondence should be addressed.
(Tel) 82-431-261-2302; (Fax) 82-431-268-2538
(E-mail) youngkim@cbucc.chungbuk.ac.kr

composition. This phenomenon is referred to as clonal turnover, which can occur within the O157 population of an individual patient. This is characterized by the appearance of new clonal genotypes and loss of old clones (9). It is possible that prophages which are known to be integrated in the O157 chromosome are partly responsible for this phenomenon. Phages that contain structural genes for *slt*I or *slt*II have been isolated from O157 strains, and their morphology, genome sizes, and RFLP have been characterized (12, 19, 21, 23).

For the purpose of generating vaccine against *E. coli* O157, we cloned the *slt*II gene encoding a shiga-like toxin type II from chromosomal DNA of *E. coli* O157 KNIH317 isolated in Korea. In this paper, we report cloning, sequencing, and analysis of *slt*II gene from chromosomal DNA of the isolate.

Materials and Methods

Bacterial strains, plasmid, and culture conditions

The bacterial strains and plasmids used or constructed in this study are presented in Table 1. *E. coli* O157 KNIH317 was isolated from a Korean patient with HUS and kindly provided by Korea National Institute of Health. The recombinant plasmids pOVT45 contains the *slt*II gene, which is responsible for cytotoxicity in Vero cells. Recombinant plasmids pOVT4501, pOVT4501, pOVT4502, pOVT 4510, and pOVT4514 were constructed from pOVT45 as shown in Table 1. All the strains carrying various recombinant plasmids were selected on Luria-Bertani (LB) agar medium containing ampicillin (50 μg/ml), tetracycline (15 μg/ml), isopropyl thio-β-D-galactoside (IPTG), and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal).

Preparation and *in vitro* manipulation of plasmid DNA

Isolation of plasmid DNA, chromosomal DNA, transformation, restriction endonuclease digestion, ligation, agarose gel electrophoresis, and other standard recombinant DNA techniques were performed as described in Sambrook *et al.* (20).

Primer design and PCR amplification

Two oligos were used as PCR primers for amplification of *slt*-related genes from the isolate. The primer (5′-CTTGAACATATATCTCAGGG-3′) is homologous to a region 148-167 nt downstream from the ATG start codon of the gene encoding the A subunit of Slt. The primer (5′-AACTCCATTAACGCCAGATA-3′) is complementary to a region 481-500 nt from the same point. Thus, these primers direct amplification of a 353-bp DNA fragment that contains partial Slt A-subunit regions. PCR amplification was carried out using a DNA thermal cycler (Bioneer Inc. Korea), with 30 cycles consisting of denaturation at 94 C for 1 min, annealing at 53°C for 1 min, and extension at 72°C for 1 min. The PCR product was purified from 1.0% agarose gel, and this is used as a probe for Southern and colony hybridization.

Cloning of *slt* gene in *E. coli* O157 KNIH317 by Southern and colony hybridization

Hybridization was carried out according to ECL (enhanced chemiluminescence, Amersham) kit and the procedure recommended by the manufacturer. Chromosomal DNA was digested with *Kpn*I, *Pst*I, and *Sal*I using the conditions as recommended by the manufacturer. After running in gel, Southern hybridization was performed using PCR product as a probe. For colony hybridization, we made mini-libraries of *E. coli* O157 KNIH317 chromosomal DNA. In brief, chromosomal DNA and pBluescript SK(+) plasmid DNA were restricted with *Kpn*I. The linearized pBluescript SK(+) DNA was treated with bacterial alkaline phosphatase for 60 min at 68°C. The DNA was extracted with phenol and chloroform and precipitated with 2 volumes of cold (-20°C) absolute ethanol. The eluted chromosomal DNA (4.5-kb *Kpn*I fragments) was precipitated by ethanol. Restricted vector and the eluted chromosomal DNAs were mixed with 1 to 5 U of T4 DNA ligase, 10 mM dithiothreitol,

Table 1. Bacterial strains and plasmids used in this study

Bacterial strain and plasmid	Relevant characteristics	Source
Strains		
E. coli O157 KNIH317		This study
E. coli XL1-Blue	*supE44hsdR17recA1endA1gyrA46thirelA1lac-F'[proAB⁺lacI^q lacZ*M15Tn10(*tet*)]	
Plasmids		
pBluescript SK(+)	Ap^r, multiple cloning site in *lacZ* α;	Stratagene Co.
pOVT45	4.5-kb *Kpn*I fragment from *E. coli* O157 KNIH317 inserted into SK(+)	This study
pOVT4501	Self-ligated small fragment of pOVT45 digested with *Pst*I	This study
pOVT4502	4.0-kb *Pst*I fragment from pOVT45 inserted into SK(+)	This study
pOVT4510	Self-ligated large fragment of pOVT45 digested with *Sma*I	This study
pOVT4511	3.3-kb *Sma*I fragment from pOVT45 inserted into SK(+)	This study
pOVT4514	0.7-kb *Sma*I fragment from pOVT4502 inserted into SK(+)	This study

and 1 mM ATP. Ligation was performed for 12 to 18 h at 16°C. The ligation mix was used to transform competent XL1-Blue cells and selected to ampicillin resistant (Amp^R) transformants. Amp^R colonies grown on the LB plates were transferred to nylon membrane and were tested for colony hybridization by using the same probe.

Expression of the cloned *slt* gene in *E. coli* cells

E. coli XL1-Blue cells harboring pOVT45 (or pOVT45-1) and pBluescrip SK(+) as controls were cultured at 37°C overnight in LB broth. Precultured cells were inoculated into the same medium and cultured at 37°C. At A_{600nm}=0.7, IPTG was added to the culture to a final concentration of 0.5 mM, and then the cells were cultured for 3 h in order to induce expression of the *slt* gene. After harvesting by centrifugation, the pellet was washed two times with phosphate buffered saline. The washed cells were sonicated 10 times for 20 sec at 4°C. After centrifugation, the supernatant was used as a crude enzyme for cytotoxicity assay in Vero cells.

Cytotoxicity assays in Vero cells

Vero cells were cultured in the Dulbeccos Modified Eagle Medium (GIBCO BRL Inc.) containing 5% fetal bovine serum, 100 μg/ml of gentamycin, and 100 U/ml of penicillin. The prepared crude enzyme was added to freshly seeded Vero cells in T-25 flask. For comparison of cytotoxicity for Vero cells, the original strain, *E. coli* O157 KNIH317 cell, was cultured in the same condition as in the case of *E. coli* XL1-Blue cells carrying pBluescript SK(+) until optical density at wavelength of 600 nm was identical for above strains.

Nucleotide sequencing

Nucleotide sequencing was determined directly from plasmids by using an Applied Biosystems automated DNA sequencer. To determine the sequence of the shiga-like toxin encoding region, DNA was subcloned into the polycloning site of the pBluescrip SK(+). Each part of both strands was sequenced. Plasmids for sequencing were purified by standard procedure using GFX™ Micro Plasmid Prep Kit (Amersham Pharmacia, USA).

Sequence analysis

The nucleotide sequence and the deduced amino acid sequence were analyzed by using the DNASIS/PROSIS (Hitachi v. 7.0). Multiple alignments were carried out on a computer using the Clustal X algorithm (25) and manually fine-tuned .

Results and Discussion

Isolation of the *E. coli* O157 KNIH317 *slt* gene

We have designed primer set and amplified the predicted size (307-bp) of partial *slt* gene from *E. coli* O157 KNIH317 using synthesized primer. Southern hybridization analyses were used to study the location of *slt* gene within the genome of *E. coli* O157 KNIH317. From Southern hybridization using the amplified PCR product as a probe, we have confirmed that the *slt* gene is located in 4.5-kb *Kpn*I fragment of the chromosome (Fig. 1. Lane 2). Therefore, we

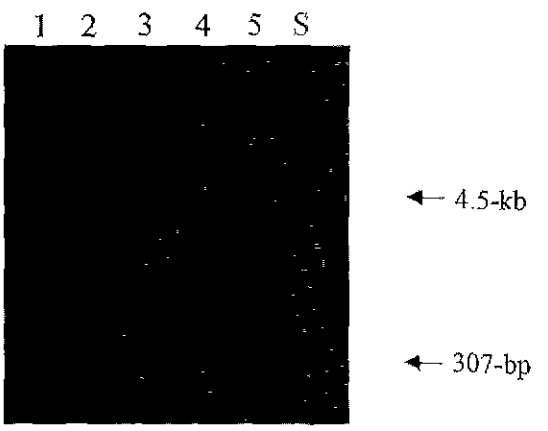

Fig. 1. Southern hybridization of *E. coli* O157 KNIH317 genomic DNA hybridizes with PCR product of *slt*-related gene. S; size marker-*Bst*EII, lane 1; *E. coli* O157 KNIH317 genomic DNA, lane 2; *E. coli* O157 KNIH317 genomic DNA digested with *Kpn*I; *Pst*I (lane 3), *Sal*I (lane 4), lane 5; PCR products. The signal was detected in 4.5-kb genomic DNA of *E. coli* O157 KNIH317 digested with *Kpn*I.

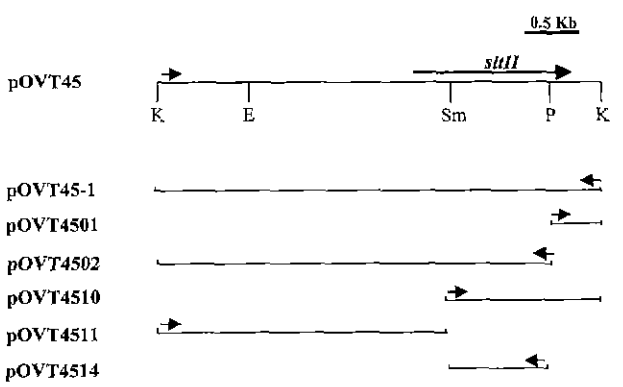

Fig. 2. Physical and genetic map of the cloned plasmid pOVT45 and its derivatives for DNA sequencing. The subclones were constructed by cloning 0.5-kb *Pst*I (pOVT4501), 4.0-kb *Pst*I (pOVT4502), 1.2-kb *Sma*I (pOVT4510), 3.3-kb *Sma*I (pOVT4511), and 0.7-kb *Sma*I (pOVT4514) fragments from pOVT45 or pOVT4502 into SK(+) vector. The arrows indicate directions of promoter in vector. Cleavage sites for the enzymes are designated as follows: K, *Kpn*I; E, *Eco*RI; Sm, *Sma*I; P, *Pst*I.

constructed mini-libraries of the isolate using *Kpn*I restriction endonuclease and pBluescript SK(+) vector as described above. Among the 500 clones of the mini-libraries, only two clones were identified positive by colony hybridization using the same probe. The isolated two strains had the same inserted fragment, but in opposite orientations. The clone which contained insert in same direction with the *lac* promoter on the vector was called pOVT45, and the other clone was called pOVT45-1.

Verocytotoxicity assay

E. coli O157 KNIH317, *E. coli* XL1-Blue (SK+), *E. coli* XL1-Blue (pOVT45,) and *E. coli* XL1-Blue (pOVT45-1) were studied for the production of verocytotoxins. The *E. coli* XL1-Blue cells harboring the recombinant plasmids (pOVT45 or pOVT45-1) had nearly the same toxicity in Vero cells (data not shown). Also, these two clones had nearly the similar toxicity in Vero cells in comparison to the wild type strain (*E. coli* O157 KNIH317), and their activity were 10

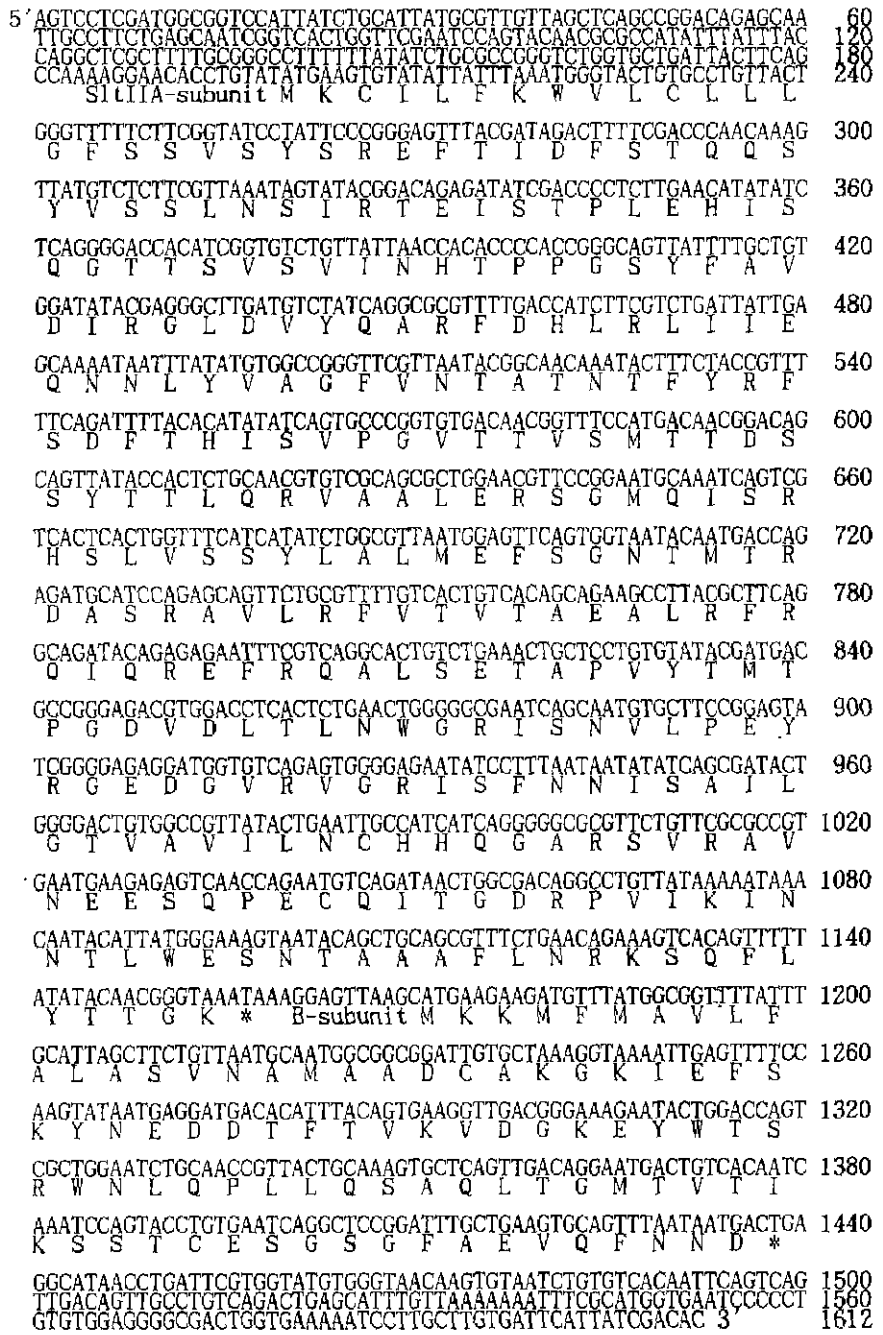

Fig. 3. Nucleotide sequence of the *slt*II gene. The predicted peptide sequences (shiga-like toxin A- and B-subunit) are also shown in one letter codes beneath the corresponding codons, and the stop codons are marked with asterisks.

Fig. 4. Alignment of nucleotide sequence of the *slt*II gene from the *E. coli* O157 KNIH317 and *E. coli* bacteriphage 933W (GenBank accession No. X07865). The asterisks indicate the different nucleotide between *E. coli* O157 KNIH317 and *E. coli* bacteriphage 933W. The arrows indicate the start and stop sites of the A- and B-subunit of shiga-like toxin II. The same nucleotide sequences are omitted.

times higher than that of negative control, *E. coli* XL1-Blue (SK+). From these results, it was concluded that the expression of inserted *slt* gene was not induced by IPTG. Therefore, the inserted fragment should have its own promoter for expression of the *slt* gene.

Nucleotide sequence and analysis of the *slt*II gene

In order to determine the sequence of the cloned *slt* region, subclones were constructed by cloning 0.5-kb *Pst*I (pOVT4501) 4.0-kb *Pst*I (pOVT4502), 1.2-kb *Sma*I (pOVT4510), 3.3-kb *Sma*I (pOVT4511) and 0.7-kb *Sma*I (pOVT4514) fragments from pOVT45 or pOVT4502 into SK(+) vector. The constructed recombinant plasmids are shown in Fig. 2. The complete nucleotide and deduced amino acid sequence of the 1.6-kb were determined (Fig. 3). Two open reading frames (ORFs) corresponding to the genes (*sltA* and *sltB*) encoding the A- and B-subunits were located from nucleotides 200-1159 and 1171-1140, respectively, within this fragment. The calculated molecular weight of the A- and B- subunit of the Slt polypeptide, based on the deduced sequence of 319 and 89 amino acids, were 35,713 and 9,874 daltons, respectively.

According to analysis by Blast E-mail server (2), the *slt* gene of *E. coli* O157 KNIH317 is highly related to the *slt*II gene of *E. coli* bacteriophage 933W (GenBank accession No. X07865), the variant *slt* gene of *E. coli* (GenBank accession No. L11079), the *slt* gene of *E. coli* (GenBank accession No. L11078), and the variant *slt*II gene of *E. coli* (GenBank accession No. X65949) have 100%, 98.4%, 93.7%, and 93.7% identities, respectively. Comparison of the nucleotide and predicted amino acid sequences of the *slt* gene from *E. coli* O157 KNIH317 with those of others in the *slt*II family revealed complete identical homology with *slt*II cloned from *E. coli* bacteriophage 933W. From this result, it can be concluded that the cloned *slt* gene belongs to *slt*II family and that the strain used in this study may be lysogeny of *E. coli* bacteriophage 933W. However, compared with the up-stream and downstream of the *slt*II gene from the *E. coli* O157 KNIH317 and *E. coli* bacteriophage 933W, the cloned

slt gene differ to three and four sites, respectively (Fig. 4). From this result, we concluded that clonal turnover may occur during the lysogenic cycle or transfer to each other's host cells.

In this study, for construction of *E. coli* O157 vaccine against diarrhea, haemorrhagic colitis and HUS, we cloned *slt*II gene of *E. coli* O157 KNIH317 isolated in Korea. Now, we are attempting overexpression of B-subunit of SltII for production of antigenicity region against *E. coli* O157.

Acknowledgment

This work was supported by KOSEF Research Grant No. 981-0503-018-2.

References

1. **Ahmed, R., C. Bopp, A. Borczyk, and S. Kasatiya.** 1987. Phage-typing scheme for *Escherichia coli* O157:H7. *J. Infect. Dis.* **155**, 806-809.
2. **Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl. Acids Res.* **25**, 3389-3402.
3. **Böhm, H. and H. Karch.** 1992. DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulse-field gel electrophoresis. *J. Clin. Microbiol.* **30**, 2169-2172.
4. **Calderwood, S.B., F. Auclair, A. Donohue-Rolfe, G.T. Keusch, and J.J. Mekalanos.** 1987. Nucleotide sequence of the shiga-like toxin genes of *Escherichia coli*. *Proc. Natl. Acad. Sci.* USA. **84**, 4364-4368.
5. **De Grandis, S., J. Ginsberg, M. Toone, S. Climie, J. Friesen, and J. Brunton.** 1987. Nucleotide sequence and promoter mapping of the *Escherichia coli*. Shiga-like toxin operon of bacteriophage H-19B. *J. Bacteriol.* **169**, 4313-4319
6. **Gannon, V.P.J., C. Teerling, S.A. Masri, and C.L. Gyles.** 1990. Molecular cloning and nucleotide sequence of another variant of the *Escherichia coli* Shiga-like toxin II family. *J. Clin. Microbiol.* **136**, 1125-1135.
7. **Hii, J.H., C. Gyles, T. Morooka, M.A. Karmali, R. Clarke, S. De Grandis, and J.L. Brunton.** 1991. Development of verotoxin 2- and verotoxin 2 variant (VT2v)-specific oligonucleotide probes on the basis of the nucleotide sequence of the B cistron of VT2v from *Escherichia coli* E32511 and B2F1. *J. Clin. Microbiol.* **29**, 2704-2709.
8. **Jackson, M.P., R.J. Neill, A.D. OBrien, R.K. Holmes, and J.W. Newland.** 1987. Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from *Escherichia coli* 933. *FEMS Microbiol. Lett.* **44**, 109-114.
9. **Karch, H., H. R ssmann, H. Schmidt, A. Schwarzkopf, and J. Heesemann.** 1995. Long-term shedding and clonal turnover of enterohemorrhagic *Escherichia coli* O157 in diarrheal disease. *J.Clin. Microbiol.* **33**, 1602-1605.
10. **Karmali, M.A.** 1989. Infection by verotoxin-producing *Escherichia coli*. *Clin. Microbiol. Rev.* **2**, 15-38.
11. **Kozlov, Y.V., A.A. Kabishev, E.V. Lukyanov, and A.A. Bayev.** 1988. The primary structure of the operons coding for *Shigella dysenteriae* toxin and temperate phage H30 shiga-like toxin. *Gene* **67**, 213-221.
12. **Newland, J.W. and R.J. Neill.** 1988. DNA probes for Shiga-like toxins I and II and for toxin-converting bacteriophages. *J. Clin. Microbiol.* **26**, 1292-1297.
13. **Ostroff, S.M., P.I. Tarr, M.A. Neill, J.H. Lewis, N. Hargrett Bean, and J.M. Kobayashi.** 1989. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. *J. Infect. Dis.* **160**, 994-998.
14. **O'Brien, A.D. and G.D. La Veck.** 1983. Purification and characterization of a *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. *Infect. Immun.* **40**, 675-683.
15. **O'Brien, A.D. and R.K. Holmes.** 1987. Shiga and shiga-like toxins. *Microbiol. Rev.* **51**, 206-220.
16. **O'Brien, A.D., G.D. La Veck, M.R. Thompson, and S.B. Formal.** 1982. Production of *Shigella dysenteriae* type 1-like cytotoxin *by Escherichia coli*. *J. Infect. Dis.* **146**, 763-769.
17. **O'Brien, A.D., J.W. Newland, S.F. Miller, R.K. Holmes, H.W. Smith, and S.B. Formal.** 1984. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Science* **226**, 694-696.
18. **Paros, M., P.I. Tarr, H. Kim, T.E. Besser, and D.D. Hancock.** 1993. A comparison of human and bovine *Escherichia coli* O157:H7 isolates by toxin genotype, plasmid profile, and bacteriophage lambda-restriction fragment length polymorphism profile. *J Infect. Dis.* **168**, 1300-1303.
19. **Rietra, P.J.G.M., G.A. Willshaw, H.R. Smith, A.M. Field, S.M. Scotland, and B. Rowe.** 1989. Comparison of Vero-cytotoxin-encoding phages from *Escherichia coli* of human and bovine origin. *J. Gen. Microbiol.* **135**, 2307-2318.
20. **Sambrook, J., E.F. Fritsch, and T. Maniatis.** 1989. Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
21. **Schmitt, C.K., M.L. McKee, and A.D. OBrien.** 1991. Two copies of shiga-like toxin II-related genes common in enterohemorrhagic *Escherichia coli* strains are responsible for the antigenic heterogenity of the O157:H- strain E32511. *Infec Immun.* **59**, 1065:1073.
22. **Scotland, S.M., H.R. Smith, and B. Rowe.** 1985. Two distinct toxins active Vero cells from *Escherichia coli* O157. *Lancet ii.* 885-886.
23. **Strockbine, N.A., L.R. Marques, J.W. Newland, H.W. Smith, R.K. Holmes, and A.D. OBrien.** 1986. Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. *Infect. Immun.* **53**, 135-140.
24. **Strockbine, N.A., M.P. Jackson, L.M. Sung, R.K. Holmes, and A.D. OBrien.** 1988. Cloning and sequencing of the genes for shiga toxin from *Shigella dysenteriae* type I. *J. Bacteriol.* **170**, 1116-1122.
25. **Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins.** 1997 The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids*

Res. **25**, 4876-4882.

26. **Wang, G., T.S. Whittam, C.M. Berg, and D.E. Berg.** 1993. RAPD (arbitrary primer) PCR is more sensitive than multilocus enzyme electrophoresis for distinguishing related bacterial strains. *Nucleic Acids Res.* **2157**, 5930-5933.
27. **Whittam, T.S., I.K. Wachsmuth, and R.A. Wilson.** 1988. Genetic evidence of clonal descent of *Escherichia coli O157:H7* associated with hemorrhagic colitis and hemolytic uremic syndrome. *J. Infect. Dis.* **157**, 1124-1133.