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Isolation and characterization of *Escherichia coli* O157:H7 and shiga toxin-converting bacteriophages from strains of human, bovine and porcine origin

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Abstract Toxin-converting bacteriophages encoding the Stx2 gene were induced from strains of *Escherichia coli* O157:H7 isolated from sewage, bovine and porcine faeces. Toxin synthesis can be stimulated by the induction of integrated toxin-converting phages from the host *E. coli* O157:H7 organism by ultra-violet (UV) exposure. The UV-mediated DNA damage of *E. coli* O157:H7 triggers a bacterial SOS response resulting in phage release. Free ranging phages outside their *E. coli* O157:H7 hosts were detected but could not be isolated directly from environmental samples such as sewage and river water. *E. coli* O157:H7 colonies carrying the genes coding for Stx2 were isolated from 1 sewage sample (0.76% of positive samples), 8 cattle faecal samples (16.67% of positive samples) and 2 pig faecal samples (14.28% of positive samples). Characterization of *E. coli* O157:H7 was done by repetitive sequence analysis using ERIC-PCR to determine the relationships between the individual *E. coli* O157:H7 strains. The ERIC-PCR analysis revealed distinct patterns for all *E. coli* O157:H7 strains with some small differences between the strains. DNA sequencing of some of the *E. coli* O157:H7 positive isolates carrying the Stx2 genes were performed confirming the amplified DNA nucleotide sequences of Stx2. Electron microscopic analysis revealed, for the first time in South Africa, that Stx2-converting phages induced from *E. coli* O157:H7 have different morphologies to that of phage lambda which was previously described. The role of the induced integrated Stx2 phages in natural environments such as river and dam water remains unclear. With the induction of Stx2-converting phages from environmental *E. coli* O157:H7 isolates, it is now possible to determine the potential of these phages to convert non-pathogenic *E. coli* strains and other enterobacteriaciae into pathogenic strains.

Keywords *E. coli* O157:H7; enterobacterial repetitive intergenic consensus; immunomagnetic separation; toxin-converting bacteriophages

Introduction

Escherichia coli (*E. coli*) serotype O157:H7, which is associated with outbreaks of haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura, embodies shiga toxins (Stx) whose genes are encoded by toxin-converting bacteriophages (phages) (Scotland *et al*., 1983; O'Brien *et al*., 1984). The toxin-converting phages are responsible for the transfer of genetic information (Stx genes and other virulence factors) between bacteria (Plunkett *et al*., 1999). Phages infecting *E. coli* O157:H7 appear to be lambdoid, which are morphologically similar to lambda (λ-type) phages (Muniesa *et al*., 1999). Lambda-type phages are temperate phages, which could show lytic or lysogenic growth characteristics. Rietra *et al*. (1989), described phages (strain 933 coding for both Stx1 and Stx2) infecting *E. coli* O157:H7 having regular hexagonal heads and short tails. *E. coli* O26 strains coding for Stx1 have elongated hexagonal heads and long tails (Rietra *et al*., 1989). However, O'Brien *et al*. (1984) reported *E. coli* strain 933 carrying the Stx1-encoding phage (933J) to be indistinguishable from Stx1 phage H19J in *E. coli* O26 strain H19.

Numerous studies have been carried out on the isolation of Stx-producing *E. coli* (STEC), especially *E. coli* O157:H7, from a variety of sources world wide (Willshaw *et al*.,

1994; Bielaszewska *et al*., 1997; Chapman *et al*., 1997; Mechie *et al*., 1997; Rahn *et al*., 1997; Itoh *et al*., 1998; Colombo *et al*., 1998; Höller *et al*., 1999; Zschöck *et al*., 2000; Müller *et al*., 2001). The prevalences of *E. coli* O157:H7 and phages carrying the gene coding for Stx2 in sewage from different countries have been investigated (Muniesa and Jofre, 1998; Höller *et al*., 1999; Muniesa and Jofre, 2000). Muniesa *et al*. (2000) demonstrated that Stx2e, a variant of Stx2, could be encoded in the genome of an infectious bacteriophage. It was found that naturally occurring phages that infect *E. coli* O157:H7 and carrying the Stx2 gene persisted more successfully in the natural environment than their host bacteria and may be the main reservoir of Stx2 in the environment (Muniesa *et al*., 1999). Osawa *et al*. (2000) demonstrated that Stx2-converting phages of different genotypes of *E. coli* O157:H7 were similar, indicating the possibility of horizontal transfer of Stx2-converting phages under some circumstances. Acheson *et al*. (1998) validated the ability of Stx1 lysogens to transduce an *E. coli* recipient strain in the murine gastrointestinal tract, giving rise to infectious virions within the host. Further studies could substantiate the possibility of Stx2 phage conversion of *E. coli* flora in the human gastrointestinal tract into pathogenic strains. In this paper we report the incidence of *E. coli* O157:H7 and Stx2 toxin-converting phages from different sources, their genetic and morphological characteristics and their comparison to each other.

Materials and methods

Sampling

Settled sewage samples (91) used in this study were collected from Daspoort, Baviaanspoort and Zeekoegat sewage purification plants and Pretoria Academic Hospital in Pretoria, South Africa. A total of 244 river and dam water samples were collected in the Pretoria region. Cattle (48) and pig (14) faecal samples were collected from the Pretoria Zoological Gardens in Pretoria and from feed lot operators near Delmas and Bronkhorstspruit in the Gauteng region of South Africa. Human faecal samples (6) that contained visible blood were obtained from the Department of Microbiology at the University of Pretoria, Pretoria, South Africa.

Bacterial strains

Shiga toxin 2 (Stx2)-positive strain *E. coli* O157:H7 (ATCC 43889) and Stx2-negative strain *E. coli* O157:H7 (ATCC 43888) were used as Stx2 positive and negative controls (Muniesa and Jofre, 1998). Stx1-positive *E. coli* C600 and Stx1-negative *E. coli* C600 were used as Stx1 positive and negative controls. *E. coli* control cultures were supplied by Prof. J. Jofre from the University of Barcelona, Spain.

Isolation of *E. coli* O157:H7 using immunomagnetic separation (IMS)

Five gram faecal samples (human, bovine and porcine) and 100 µl sewage samples were inoculated in 50 ml of buffered peptone-saline water (Oxoid, CM509) supplemented with Vancomycin (8 $mg.l^{-1}$), Cefixime (0.05 $mg.l^{-1}$) and Cefsulodin (10 $mg.l^{-1}$) (VCC) antibiotic solution (MAST® Diagnostics) to inhibit the growth of interfering bacteria. River water samples (100 ml) were filtered through 0.45 µm Gelman GN-6 Metricel filter membranes (Prod no. 66191) before enrichment. The suspensions were incubated in a shaker incubator (Hub-O-Mat) for 6 h at 37°C while rotating at 100 rpm. IMS was performed on the enrichment cultures as described by the product manufacturer (Dynal® product brochure, 1995). The final bead-bacteria complexes were resuspended in 100 µl washing buffer (PBS-Tween). After IMS, 100 µl of faecal IMS concentrates were transferred to Cefixime-tellurite Sorbitol-MacConkey agar (CT-SMAC) (Oxoid). *E. coli* O157:H7 strains produced typical colourless colonies on CT-SMAC after 24 h of incubation at 37°C.

Suspect *E. coli* O157:H7 colonies were individually tested for agglutination using a commercial *E. coli* O157 slide agglutination kit with antisera against *E. coli* O157 (Mast Assure, Mono Factor O157, code: M12030). All colonies were biochemically confirmed as *E. coli* by their ability to produce indole from tryptophan using Kovac's reagent (ISO, 2001).

Molecular detection of *E. coli* O157:H7

Presumptive *E. coli* O157:H7 colonies obtained from CT-SMAC were examined for the presence of the integrated genes coding for Stx1, Stx2, *eae*A and the additional plasmid-mediated enterohaemolysin which is present in almost all *E. coli* O157:H7 organisms. The bacterial colonies were suspended in 500 µl distilled water and boiled in 1.5 ml microcentrifuge tubes for 10 min at 99°C. The samples were centrifuged at 10,000 g (Eppendorf Centrifuge 5402) for 1 min and 10 µl volumes of the supernatant containing genomic DNA were amplified. Oligonucleotide primers (synthesized by Sigma-Genosys Ltd., London Road, Pampisford, Cambridgeshire, CB2 4EF, UK) specific for Stx1 (VT1) (Pollard *et al*., 1990), Stx2 (VT2) (Pollard *et al*., 1990), *eae*A (Gannon *et al*., 1993) and the haemolysin plasmid (Fratamico *et al*., 1995) were used in the polymerase chain reaction (PCR) (Table 1).

The PCR cycle for all reactions consisted of an initial 5 min DNA denaturation cycle at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C (Pharmacia LKB Thermocycler). The amplicons (20 µl aliquots from each amplification) were detected with gel electrophoresis using a 2% agarose gel (SeaKem® LE) stained with ethidium bromide (Sigma). A 100 base pair DNA (Promega) was used as molecular size marker.

Sequencing of genome integrated Stx2

Sequencing of PCR products of some *E. coli* O157:H7 positive isolates carrying the Stx2 genes were carried out according to the T7 Sequenase$_{v2.0}$ protocol described by Amersham Life Science (prod. nr. US70170). Sequencing was performed to confirm the amplification of Stx2. Dimethyl sulfoxide (DMSO) was added to the annealing template to enhance primer specificity and amplification. Forward toxin sequences of the 346 bp fragment were compared to the gene sequence of Stx2 on GenBank (AF291819.1; GI = 9858182).

Table 1 Primer sequences and predicted sizes of PCR amplified products for the detection of ERIC1R, ERIC2, EHEC O157, the haemolysin plasmid and Stx (VT)-specific genes of *E. coli* O157:H7

Primer	Oligonucleotide sequence (5′–3′)	Target(s)	Size of amplified product (base pairs)	Reference
ERIC 1R	CACTTAGGGGTCCTCGAATGTA			
ERIC 2	AAGTAAGTGACTGGGGTGAGCG	Conserved repeated sequences	50–3000	De Bruijn (1992)
VT1a	GAAGAGTCCGTGGGATTACG	Stx 1		
VT1b	AGCGATGCAGCTATTAATAA		130	Pollard *et al.* (1990)
VT2a	TTAACCACACCCACGGCAGT			
VT2b	GCTCTGGATGCATCTCTGGT	Stx2	346	Pollard *et al.* (1990)
EHEC 1*	CAGGTCGTCGTGTCTGCTAAA			
EHEC 2*	TCAGCGTGGTTGGATCAACCT	*eae*A	1087	Gannon *et al.* (1992)
EHEC/P1#	ACGATGTGGTTTATTCTGGA			
EHEC/P2#	CTTCACGTCACCATACATAT	60-MDa haemolys in plasmid	166	Fratamico *et al.* (1995)

* = EHEC genes specific for *E. coli* O157

= Haemolysin plasmid

Primers were obtained from Sigma-Genosys Ltd. London Road, Pampisford, Cambridgeshire, CB2 4EF, UK

Repetitive sequence analysis

In addition, genomic DNA extraction and purification of environmental *E. coli* O157:H7 isolates for repetitive sequence analysis (RSA) was performed as follows: *E. coli* O157:H7 isolates were grown on LB agar and several colonies were dispersed in 4.5 ml of sterile distilled water. The optic density of the suspensions was measured (wavelength 620 nm) to obtain a standardised number of cells. The formula used to calculate the number of *E. coli* O157:H7 cells was $V_{(ml)} = 0.2/OD_{620}$. The cell suspensions were centrifuged (Sorvall Super T21) at 13,000 g for 5 min at 4°C. Supernatants were discarded and excess media blotted away. Volumes of 100 µl of ultra high quality (UHQ) water and Tris-HCl (10 mM, pH = 8.2) were added to the cell pellets. Proteinase K (1 $mg.ml^{-1}$) was added to the suspensions and incubated at 55°C overnight. Boiling the samples at 99°C for 10 min inactivated the proteinase K. Cell lysates were stored at –20°C. The following was added to each 90 µl ERIC-PCR mixture: 12 µl of 10x Mg-free buffer (Promega); 6 µl of 10 mM $MgCl_2$ (Promega); 1.5 µl of dNTP mixture (Promega); 0.2 µl of Taq DNA Polymerase (Promega); 25 pmol of each ERIC1R and ERIC2 primer (Sigma-Genosys Ltd.) (Table 1) and 10 µl of purified template DNA. The PCR was performed with a Hybaid OmniGene Thermocycler. The use of ERIC sequences and PCR was based on the study performed by De Bruijn (1992). The PCR cycle consisted of one cycle at 95°C for 7 min, followed by 35 cycles of 1 min at 94°C, 1 min at 40°C and 8 min at 65°C and a final cycle of 16 min at 65°C (Hybaid OmniGene Thermocycler). The amplicons (20 µl aliquots from each amplification) were detected with polyacrylamide gel electrophoreses (PAGE) using a 4% acrylamide suspension stained with ethidium bromide (Sigma). Marker XIV (Roche) was used as a 100 bp molecular size marker.

***E. coli* O157:H7 phage induction**

Phages were induced from *E. coli* O157:H7 cultures, carrying the Stx2 genes, isolated from sewage, cattle and pigs. Induction of phages using ultraviolet (UV) light from the positive *E. coli* O157:H7 isolates was done according to methods described by O'Brien *et al.* (1984). The *E. coli* O157:H7 strains were inoculated in 5 ml of LB medium with a single colony of *E. coli* O157:H7 and incubated at 37°C with shaking (100 rpm) overnight. These culture suspensions were diluted 1:20 in 100 ml of LB medium, incubated at 37°C with shaking (100 rpm) for 2–3 hours until the beginning of the exponential growth phase ($OD_{600} = 0.1$). The cells were harvested by centrifugation and supernatants discarded. The *E. coli* O157:H7 cells were resuspended in a 10 ml solution containing 10 mM $MgCl_2$ and 10 mM $CaCl_2$. The time of UV exposure quoted by O'Brien *et al.* (1984) proved to be too long and killed all cells. A killing curve experiment was carried out to establish optimum radiation time and to standardise the induction procedure. According to this experiment all the solutions were exposed to UV light for 2 seconds. After UV exposure all manipulations were carried out in the dark to prevent photo repair of the UV induced damage. The UV exposed cells were diluted 1:10 in LB medium. These suspensions were incubated overnight at 37°C with shaking (Hub-O-Mat) (100 rpm). Phages were extracted by chloroform treatment (1:10) for 30 min, vortexed and centrifuged for 15 min at 2,000 g. Supernatants were filter sterilised through 0.22 µm Millex GV (Millipore) filter-units. Phage production was confirmed using the double agar layer plaque assay as described below. All phage lysates were stored at –70°C for phage characterisation.

Phage enumeration

E. coli O157:H7 phages were enumerated by the double agar layer procedure using Modified Scholtens Agar (MSA) (Muniesa and Jofre, 1998). Stx2-negative strain *E. coli* O157:H7 (ATCC 43888) was used as host culture, incubated at 37°C until an appropriate

logarithmic growth phase for phage enumeration was obtained. A total of 1 ml of each direct sample and dilutions with phosphate-buffered saline solution (PBS) was inoculated with the host culture, mixed with semisolid agar and poured onto solid agar plates. After settling of the semisolid agar mixture, plates were incubated overnight at 37°C.

Free-ranging phage enrichment cultures

Sewage and environmental water samples (30 ml) were centrifuged at 3,000 g (Beckman GS-6R) for 15 min to remove debris. This step was followed by the filtration of the supernatants through 0.22 µm pore-size low-protein-binding Millex-GV membrane filter-units (Millipore Corporation) to eliminate bacteria but allowing phages to pass through. The filtered samples were treated with DNase (10 $u.ml^{-1}$) to eliminate free DNA in suspension. Bacteriophage enrichment cultures were prepared in Modified Scholtens broth (MSB) as described by Muniesa and Jofre (1998). Sample volumes of 10 ml were added to 100 ml cultures of Stx-negative *E. coli* O157:H7 (ATCC 43888) at the logarithmic growth phase. MSB was added to a final volume of 250 ml (Muniesa and Jofre, 1998). Enrichment cultures were shake incubated (Hub-O-Mat) overnight at 37°C (100 rpm). Volumes of 45 ml were centrifuged (Beckman GS-6R) at 3,000 g for 30 min and the supernatants were filtered through the same 0.22 µm pore-size low-protein-binding Millex-GV membrane filter-units. Phage supernatants were stored at –70°C for phage enumeration, phage DNA extraction and electron microscopy.

Free-ranging phage DNA extraction and amplification for Stx1 and Stx2

Phage DNA was extracted from liquid culture suspensions obtained after phage enrichment using the High Pure™ Lambda Isolation Kit as described by the product (Roche product nr. 2012 871). This extraction kit is specific for the purification of Lambda DNA from liquid cultures and from plate lysates. The eluted DNA suspensions were used in PCR amplification. The same Stx1- and Stx2 primers and amplification procedures were followed as described for the detection of integrated Stx1 and Stx2.

Electron microscopy

Phage preparations for electron microscopy (EM) were prepared as follows: the Stx2-phage plaque selected from the MSA plate was inoculated in 8 ml of LB-medium containing 1–2 ml of logarithmic growth phase *E. coli* O157:H7 (ATCC 43888 – Stx2 negative) host culture. This suspension was incubated overnight at 37°C without shaking, mixed with chloroform (1:10) and centrifuged at 1,000 g for 15 min. The phage supernatant was transferred to a 1.5 ml microcentrifuge tube and centrifuged at 100,000 g for 1 h, washed with 1.5 ml of UHQ water and again centrifuged at 100,000 g for 1 h. The supernatant was discarded and the phage pellet mixed with 20–50 µl of 2% phosphotungstic acid (PTA; pH 6.8) and placed on a 400-mesh Formvar carbon-coated grid (Taylor *et al*., 1993). The dried grid was examined in a Philips 300 EM.

Results

Isolation of *E. coli* O157:H7

A total of 91 sewage-, 244 river water-, 6 human-, 48 bovine-, and 14 porcine faecal samples were analysed for the presence of *E. coli* O157:H7 using IMS and molecular techniques. The following samples tested positive for *E. coli* O157:H7: 3 strains from 1 sewage sample (0.76% sewage samples), 26 strains from 6 bovine samples (12.50% bovine samples) and 3 strains from 2 porcine samples (14.29% porcine samples). None of the human faecal samples or river water samples were positive for *E. coli* O157:H7. All the positive *E. coli* O157:H7 strains contained the genes coding for Stx2, *eae*A and the

enterohaemolysin plasmid. These positive *E. coli* O157:H7 isolates were used for phage induction procedures.

Partial sequencing of Stx2

The nucleotide sequence of *E. coli* O157:H7 Stx2 as described by Chen *et al.* (2000) were compared to the sequences of some of the Stx2-positive *E. coli* O157:H7 strains obtained in this study. The 346 bp nucleotide sequences of 8 environmental isolates (2 sewage, 5 bovine and 1 porcine) showed similar sequences as the Stx2 sequence (located within the 219–564 bp region of the Stx2 gene) of GenBank (AF291819.1; GI: 9858182), confirming the amplification of Stx2.

Repetitive sequence analysis

RSA was used to detect highly conserved short intergenic repeated sequences in the *E. coli* O157:H7 genome (De Bruijn, 1992). Total chromosomal DNA was extracted from 10 positive *E. coli* O157:H7 environmental isolates which include: 2 sewage (DP2(2) and DP2(4)); 6 cattle (F3(1)25, F2(2)7, F2(3)6, F4(3)1, F5(5)12 and DB6(4)); and 2 pigs (DV1(1) and DV5(2)). The 3 reference strains used were Stx2-positive-*E. coli* O157:H7 (ATCC 43889), Stx2-negative *E. coli* O157:H7 (ATCC 43888) and *E. coli* C600. In this study ERIC-PCR generated multiple amplification products ranging between 50 bp and 3,000 bp (Figure 1). The ERIC-PCR analysis revealed distinct patterns for all *E. coli* O157:H7 strains. Minor differences in pattern were observed in the 600–800 bp region. The two sewage sample isolates (Dp2(2) and Dp2(4)) showed an additional amplification product at the ± 680 bp region (Figure 1, lanes 1 and 8) which correlated with a non-characterised non-O157 *E. coli* pig isolate (DV5(2)) (Figure 1, lane 9) but not with the bovine and porcine isolates. One bovine sample isolate (F4(3)1) (Figure 1, lane 6) lacked an amplification product located at ± 780 bp which is present in all the other *E. coli* O157:H7 isolates. *E. coli* C600 and DV5(2) (non-O157 isolates) showed differences when compared to the *E. coli* O157:H7 isolates. ERIC-PCR demonstrated differences between the Stx2-negative (ATCC 43888) and Stx2-positive (ATCC 43889) *E. coli* O157:H7 controls. The environmental isolates shared a similar ERIC-PCR pattern as the Stx2-negative *E. coli* O157:H7 but some differences were observed for the Stx2-positive strain.

Free-ranging phage detection

Seventy-eight sewage samples were analysed with PCR for phages carrying the genes coding for Stx1 and Stx2. The average DNA yield after DNA extraction of phage enrichment cultures was between 20 and 160 ng.μl^{-1}. The mean number of phages infecting *E. coli* O157:H7 found in sewage was 3.05×10^2 pfu.ml^{-1}. Two samples (Daspoort West sewage intake and Hartebeespoort Dam) (2.56% of all samples) confirmed the presence of phages carrying the genes coding for Stx2 with PCR. Although they were confirmed positive with PCR these phages could not be isolated directly from the original 2 samples by using the standard double agar layer plaque assay described by Muniesa and Jofre (1998).

E. coli O157:H7 phage induction

Environmental strains of *E. coli* O157:H7 were exposed to ultraviolet radiation at different time intervals to establish the optimal phage release conditions. From a total of 32 strains of *E. coli* O157:H7 subjected to the induction procedure only 5 strains (15.62%) produced phages after induction with UV. Phage titres of phages obtained from the 5 induced *E. coli* O157:H7 organisms were increased with overnight incubation of phage suspensions in a host *E. coli* O157:H7 culture lacking the Stx2 genes. Plaques obtained from all induced cultures (Stx2 positive) were small (<1 mm in diameter). Sergeant (1998) demonstrated the

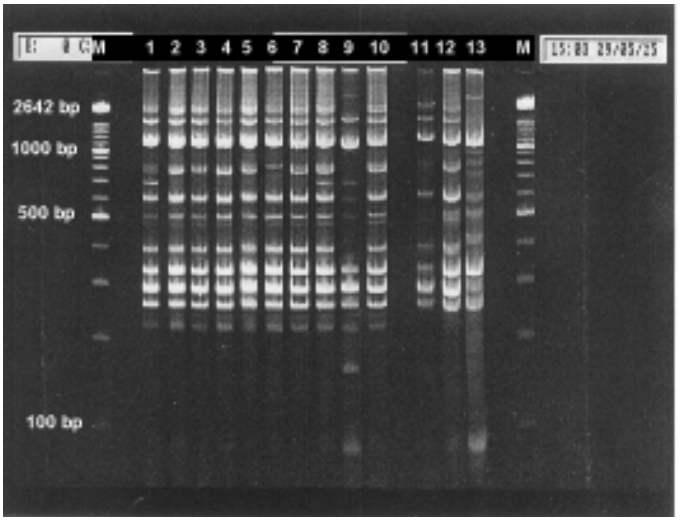

Figure 1 Graphic representation of the repetitive sequence analysis of *E. coli* O157:H7 isolates using gel electrophoresis. Lanes: 1. Sewage DP2(4); 2. Cattle F3(1)25; 3. Pig DV1(1); 4. Cattle F2(2)7; 5. Cattle F2(3)6; 6. Cattle F4(3)1; 7. Cattle F5(5)12; 8. Sewage DP2(2); 9. Pig DV5(2); 10. Cattle DB6(4); 11. *E. coli* O157:H7 Stx2-positive control (ATCC 43889); 12. *E. coli* O157:H7 Stx2-negative control (ATCC 43888); 13. *E. coli* C600. M = 100 bp marker

same phenomenon of small plaques and reduced burst sizes when compared to wild-type or laboratory strains of phage λ.

Electron microscopy

EM was performed on phages isolated from sewage, cattle and pigs. Stx2-converting phages induced from *E. coli* O157:H7 isolated from Daspoort West Intake sewage (Dp2(4)) had elongated heads and a long non-contractile tail structure. Rietra *et al*. (1989) described Stx1-converting phages induced from *E. coli* O26:H11, strain H19 and E40877 with a similar structure to phages induced from Dp2(4) in this study. Phages from *E. coli* strain 933, serotype O157:H7, which codes for the production of Stx1 had lambda-like phage morphology (regular hexagonal heads with short non-contractile tails). *E. coli* O157:H7 strain 933 coding for Stx2 had the same morphology as strain 933 coding for Stx1.

Discussion and conclusions

Screening of human and animal stool specimens for *E. coli* O157:H7 has increased due to the severe effect of illness in affected patients (Griffin, 1998). Griffin (1998) reported that 0.4% of 30,000 human stool specimens yielded *E. coli* O157:H7 while Höller *et al.* (1999) reported a 3.2% *E. coli* O157:H7 detection rate in municipal sewage. Zschöck *et al*. (2000) studied the occurrence of EHEC in healthy dairy ruminants (13,552 *E. coli* colonies from 726 cows, 28 sheep and 93 goats) and found 0.66% of *E. coli* strains investigated positive for the genes coding for Stx1, 0.47% positive for Stx2 and 0.42% positive for Stx1 and Stx2. None of these isolates belonged to the *E. coli* O157:H7 serogroup (Zschöck *et al*., 2000). Beutin (1998) summarized that the percentage detection rate of Stx-producing *E. coli* O157:H7 among 567 pig stool specimens examined was <0.2%. Henton and Engelbrecht (1997) described 10 *E. coli* O157 (H-type unknown) organisms isolated in South Africa over a period of 20 years (1971 to 1991).

In this study we found a low percentage of *E. coli* O157:H7 positive isolates in

municipal- and industrial sewage, although the detection rate of the organism in cattle and pigs was significantly higher when compared to the previously mentioned studies (Henton and Engelbrecht, 1997; Beutin, 1998; Zschöck *et al*., 2000). This is the first study in South Africa in which the ERIC-PCR was used to compare different strains of *E. coli* of the same O157:H7 serotype. ERIC-PCR analysis is extremely sensitive and this study proved that it can detect small differences between different strains of the same bacterial serotype. Direct detection of phages in sewage was low when compared to another study performed by Muniesa and Jofre (2000), where free-ranging phages were detected in South African sewage but could not be directly isolated. This could be due to different primer pairs used or the lack of an extra nested-PCR step.

UV induction experiments of *E. coli* O157:H7 revealed that the maximum number of Stx2-converting phages was released when the bacterial cells were exposed to UV for 2 seconds and thereafter the phage titre gradually decreased when exposed for longer intervals. This differs from the findings of O'Brien *et al*. (1984). Some strains were subjected to Mitomycin C but UV-radiation proved superior when phage titres were compared after induction. DNA damage, caused by the UV radiation, triggers a bacterial SOS response which increases phage production and expression of Stx and other phage genes (Yoh and Honda, 1997; Kimmit *et al*., 2000). The difficulty to induce Stx2-converting phages from the environmental strains of *E. coli* O157:H7 was confirmed by the low titres obtained after UV radiation. Sergeant (1998) described that most Stx-positive *E. coli* strains failed to produce phages and therefore presumably harboured defective prophages. The same conclusion could be reached with the results obtained in this study, a phenomenon commonly encountered among toxigenic phages (Saunders *et al*., 1999). Stx2-converting phages induced from environmental *E. coli* O157:H7 isolates revealed for the first time in South Africa that these phages have different morphologies to the previously described phage lambda.

In summary, the data obtained in this study (the low numbers of *E. coli* O157:H7 bacteria isolated from humans and municipal sewage) support the findings of the WHO (1997) that the incidence of *E. coli* O157:H7 infections among humans in South Africa is low when compared to countries in the northern hemisphere. An interesting observation from the data obtained in this study is the high percentage of *E. coli* O157:H7 organisms isolated from healthy cattle and pigs, when only 10 isolates of *E. coli* O157 have been isolated in South Africa from pigs with haemorrhagic colitis in the past 20 years (WHO, 1997). Data obtained from phage induction experiments seem to correspond with other studies (Sergeant, 1998; Jofre, 2001), which indicate a low induction success rate. For future research these Stx2-converting phages induced from environmental *E. coli* O157:H7 isolates will be used to determine host specificity and toxin conversion among other members of the enterobacteriaciae family.

References

Acheson, D.W., Reidl, J., Zhang, X., Keusch, G.T., Mekalanos, J.J. and Waldor, M.K. (1998). *In vivo* transduction with shiga toxin 1-encoding phage. *Infection and Immunity* **66**, 4496–4498.

Bielaszewska, M., Janda, J., Bláhová, K., Minaríková, H., Jíková, E., Karmali, M.A., Laubová, J., Sikolová, J., Preston, M.A., Khakhria, R., Karch, H., Klazarová, H. and Nyc, O. (1997). Human *Escherichia coli* O157:H7 infection associated with the consumption of unpasteurized goat's milk. *Epidemiology and Infection* **119**, 299.

Chapman, P.A., Siddons, C.A., Cerdan Malo, A.T. and Harkin, M.A. (1997). A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiology and Infection* **119**, 245–250.

Chen, L.-M., Pan, T.-M. and Su, Y.-C. (2000). Identification and Characterization of Enterohemorrhagic *Escherichia coli* O157:H7 by using Multiplex PCR Assays for *hly*A, *eae*A, stx1, stx2, fliC, and rfbO157. *Journal of Clinical Microbiology*. In press.

Colombo, S., Pacciarini, M.L. and Fusi, P. (1998). Isolation of a new phenotypic variant of *E coli* O157:H7 from food. *Veterinary Record* **142**, 144–145.

De Bruijn, F.J. (1992). Use of Repetitive (Repetitive Extragenic Palindromic and Enterobacterial Repetitive Intergenic Consensus) Sequences and the Polymerase Chain Reaction to Fingerprint the Genomes of *Rhizobium meliloti* Isolates and Other Soil Bacteria. *Applied and Environmental Microbiology* **58**, 2180–2187.

Dynal® product brochure (1995). Dynabeads® anti-E. coli O157. Dynal A.S, Oslo, Norway.

Fratamico, P.M., Sackitey, S.K., Wiedmann, M. and Deng, M.Y. (1995). Detection of *Escherichia coli* O157:H7 by multiplex PCR. *Journal of Clinical Microbiology* **33**, 2188–2191.

Gannon, V.P.J., Rashed, M., King, R.K. and Thomas, E.J.G. (1992). Detection and characterization of the *eae* gene of shiga-like toxin-producing *Escherichia coli* using polymerase chain reaction. *Journal of Clinical Microbiology* **31**, 1268–1274.

Henton, M.M. and Engelbrecht, M.M. (1997). *Escherichia coli* serotypes in pigs in South Africa. *Onderstepoort Journal of Veterinary Research* **64**, 175–187.

Höller, C., Koshinsky, S. and Witthuhn, D. (1999). Isolation of enterohaemorrhagic *E. coli* from municipal sewage. *Lancet* **353**, 2039.

ISO (2001). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157. ISO/FDIS 16654.

Itoh, Y., Sugita-Konishi,Y., Kasuga, F., Iwaki, M., Hara-Kudo, Y., Saito, N., Noguchi, Y., Konuma, H. and Kumagai, S. (1998). Enterohaemorrhagic *E. coli* present in radish sprouts. *Applied and Environmental Microbiology* **64**, 1532–1535.

Jofre, J. (2001). Personal communication.

Kimmit, P.T., Harwood, C.R. and Barer, M.R. (2000). Toxin gene expression by shiga toxin-producing *Escherichia coli*: the role of antibiotics and the bacterial SOS response. *Emerging Infectious Diseases* **6**(5), 2000.

Mechie, S.C., Chapman, P.A. and Siddons, C.A. (1997). A fifteen month study of *Escherchia coli* O157:H7 in a dairy herd. *Epidemiology and Infection* **118**, 17–25.

Müller, E.E., Ehlers, M.M. and Grabow, W.O.K. (2001). The Occurrence of *E. coli* O157:H7 in South African Water Sources Intended for Direct and Indirect Human Consumption. *Water Research* **35**, 3085–3088.

Muniesa, M. and Jofre, J. (1998). Abundance in sewage of bacteriophages that infect *Escherichia coli* O157: H7 and that carry the shiga toxin 2 gene. *Applied and Environental Microbiology* **64**, 2443–2448.

Muniesa, M. and Jofre, J. (2000). Occurrence of phages infecting *Escherichia coli* O157: H7 carrying the Stx 2 gene in sewage from different countries. *FEMS Microbiology Letters* **183**, 197–200.

Muniesa, M., Lucena, F. and Jofre, J. (1999). Comparative Survival of Free Shiga Toxin 2-Encoding Phages and *Escherichia coli* Strains outside the Gut. *Applied and Environmental Microbiology* **65**, 5612–5618.

Muniesa, M., Recktenwald, J., Bielaszewska, M., Karch, H. and Schmidt, H. (2000). Characterization of a shiga toxin 2e-converting bacteriophage from an *Escherichia coli* strain of human origin. *Infection and Immunity* **68**, 4850–4855.

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 *E.. coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Science* **226**, 694–696.

Osawa, R., Iyoda, S., Nakayama, S.I., Wada, A., Yamai, S. and Watanabe, H. (2000). Genotypic variations of Shiga toxin-converting phages from enterohaemorrhagic *Escherichia coli* O157:H7 isolates. *Journal of Medical Microbiology* **49**, 565–574.

Plunkett, G. 3rd., Rose, D.J., Durfee, T.J. and Blattner, F.R. (1999). Sequence of Shiga toxin 2 phage 933W from *Escherichia coli* O157:H7: Shiga toxin as a phage late-gene product. *Journal of Bacteriology* **181**, 1767–1778.

Pollard, D.R., Johnson, W.M., Lior, H., Tyler, S.D. and Rozee, K.R (1990). Rapid and specific detection of verotoxin genes in Escherichia coli by the polymerase chain reaction. *J. Clin. Microbiol.* **28**, 540–545.

Rahn, K., Renwick, S.A., Johnson, R.P., Wilson, J.B., Clarke, R.C., Alves, D., McEwan, S., Lior, H. and Spika, J. (1997). Persistance of *Escherichia coli* O157:H7 in dairy cattle and the dairy farm environment. *Epidemiology and Infection* **119**, 251–259.

Rietra, P.J.G.M., Wilshaw, G.A., Smith, H.R., Field, A.M., Scotland, S.M. and Rowe, B. (1989). Comparison of Vero-cytotoxin-encoding Phages from *Escherichia coli* of Human and Bovine Origin. *Journal of General Microbiology* **135**, 2307–2318.

Saunders, J.R., Sergeant, M.J., McCarthy, A.J., Mobbs, K.J., Hart, C.A., Marks, T.S. and Sharp, R.J. (1999). Genetics and molecular ecology of *Escherichia coli* O157. In: *E. coli* O157 in farm animals. Eds. C.S. Steward and H.J. Flint. CAB International.

Scotland, S.M., Smith, H.R., Wilshaw, G.A. and Rowe, B. (1983). Verocytotoxin production in strain of *Escherichia coli* is determined by genes carried on bacteriophage. *Lancet* **2**, 216.

Sergeant, M.J. (1998). Molecular biological characterization of verotoxigenic bacteriophages in *Escherichia coli*. PhD thesis, University of Liverpool.

Taylor, M.B., Schildhauer, C.I., Parker, S., Grabow, W.O.K., Jiang, X., Estes, M.K., and Cubitt, W.D. (1993). Two successive outbreaks of SRSV-associated gastroentiritis in South Africa. *Journal of Medical Virology* **41**, 18–23.

Willshaw, G.A., Thirlwell, J., Jones, A.P., Parry, S., Salmon, R.L. and Hickey, M. (1994). Vero cytotoxin-producing *Escherichia coli* O157 in beefburgers linked to an outbreak of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome in Britain. *Letters in Applied Microbiology* **19**, 304–307.

WHO (1997). Consultations and Workshops. Prevention and Control of Enterohaemorrhagic *Escherichia coli* (EHEC) Infections. Report of a WHO Consultation, Geneva, Switzerland. 8 April–1May.

Yoh, M. and Honda, T. (1997). The stimulating effect of fosfomycin, an antibiotic in common use in Japan, on the production/release of verotoxin-1 from enterohaemorrhagic *Escherichia coli* O157:H7 *in vitro*. *Epidemiology and Infection* **119,** 101–103.

Zschöck, M., Hamann, H.P., Kloppert, B. and Wolter, W. (2000). Shiga-toxin-producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properties. *Letters in Applied Microbiology* **31**, 203–208.