Shiga toxin encoding bacteriophages and horizontal gene transfer in *Escherichia coli* O157

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Certificate of Authorship/Originality

I certify that the work presented in this thesis has not previously been submitted for a degree, nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the written preparation of the thesis, and all experimental work associated with it, has been carried out solely by me, unless otherwise indicated.

Finally, I certify that all information sources and literature used are acknowledged in the text.

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Abbreviations

Abbreviation	Meaning
att	Attachment site
bp	Base pair
BLAST	Basic Local Alignment Search Tool
CDS	Coding sequence
Cm	Chloramphenicol
Cfu	Colony forming unit
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DNA	Deoxyribonucleic acid
DNTP	Deoxynucleotide
ELISA	Enzyme-linked immunosorbent assay
G+C	Guanine+Cytosine
gDNA	Genomic DNA
HGT	Horizontal gene transfer
HUS	Haemolytic uremic syndrome
IR	Inverted repeat
IS	Insertion sequnce
Km	Kanamycin
kb	Kilo base
L	litre
LB	Luria broth
LEE	Locus of Enterocyte Effacement
M	Molar
mM	Millimolar
Mb	Mega base
ml	Millilitre
µl	Mircolitre
MITE	Miniature Inverted-repeat Transposition Element
MLEE	Multi-Locus Enzyme Electrophoresis
MLVA	Multiple- Locus Variable Number Tandem Repeat
MMC	Mitomycin C
MOI	Multiplicity of infection
nm	Nanometre
NCBI	National Centre for Biotechnology information
PCR	Polymerase chain reaction
Phage	Bacteriophage
pfu	Plaque forming unit
qPCR	Quantitative Polymerase chain reaction
RNA	Ribonucleic acid
R-M	Restriction modification
Rif	Rifampicin
Sm	Streptomycin
SMRT	Single Molecule Real Time
SNP	Single Nucleotide Polymorphism
Stx	Shiga toxin
STEC	Shiga toxigenic *E. coli*

Abbreviation	Meaning
T3SS	Type 3 Secretion System
TA	Toxin-antitoxin
TEM	Transmission electron microscopy
tRNA	Transfer ribonucleic acid
WT	Wildtype

Research outputs

Publications

Sim, E. M., P. S. Chandry, S. A. Beatson, K. S. Gobius & N. K. Petty (2016). Insights into the emergence of pathogens by genomic analysis of Shiga toxin 1 encoding bacteriophages originating from clinical Australian *Escherichia* coli O157 isolates (Manuscript in preparation).

Sim, E. M., K. S. Gobius & N. K. Petty (2016). Insights into the regulation of Australian Stx1 and Stx2c prophages in STEC O157 (Manuscript in preparation)

Conference proceedings

Sim, E.M., P. S. Chandry, S. A. Beatson, K. S. Gobius & N. K. Petty (January 2015) **POSTER.** Characterisation of Shiga Toxin 1 phages reveal insights for formation of new pathogens. *Bacteriophages 2015*, London, United Kingdom

Sim, E.M., P. S. Chandry, S. A. Beatson, N. K. Petty & K. S. Gobius (September 2013) **ORAL PRESENTATION.** Characterisation of novel Shiga toxin 1 encoding bacteriophages originating from Australian *E. coli* O157 isolates**.** *BacPath 12,* Brisbane, Australia.

Sim, E.M., N. K. Petty, T. J. Wells, M. Leitner, M. J. Sullivan, S. A. Beatson & K. S. Gobius (July 2012) **POSTER.** Phage mediated transfer of *Escherichia coli* O157 initimin gene (*eae*) encoded by the Locus of Enterocyte Effacement, *40th Annual Meeting of the Australian Society of Microbiology*, Brisbane, Australia.

Book Chapter

Turner, D., J. M. Sutton, D. M. Reynolds, **E. M. Sim** & N. K. Petty (2015) Visualisation of phage genomic data: comparative genomics and publication-quality diagrams. In *Bacteriophages: Methods & Protocols*. A. M. Kropinski & M. R. J. Clokie (eds). Humana Press (Submitted).

Abstract

Shiga toxigenic *Escherichia coli* (STEC), most notably serotype O157, is a food borne pathogen of global public health concern. The progression to a severe disease state following an STEC infection is associated with the production of Shiga toxin (Stx). The ability to produce Stx is conferred upon STEC strains by Stx–encoding bacteriophages (Stx phages), which infect and integrate into the host bacterial genome. These phages carry either stx_1 or stx_2 genes which encode two immunologically distinct toxins with similar biological functions. However, not all STEC O157 strains are equally pathogenic as Australian STEC O157 strains, are associated with lower incidence of clinical disease than strains from other countries. This led to the aim of this thesis, which was to investigate how Stx phages differentially influence the virulence of STEC O157 strains

The characterisation of two Stx1 phages, originating from clinical Australian STEC O157 strains, revealed that these phages are morphologically and genomically similar to Stx2a phages. It was also observed that the bacterial host genetic background could influence toxin production. Genomic analysis revealed that these phages can potentially induce a translational frameshift with two overlapping tail-coding sequences with different host recognition domains, which may account for the broad host range of Stx phages leading to the emergence of new Stx producing pathogens. In addition, *in-silico* analysis also revealed a possible mechanism on how the phage obtained the *stx* gene by means of a Miniature Inverted Transposable element.

Stx prophages from three non-clinical Australian STEC O157 isolates were also characterised in this thesis. Two STEC O157 strains harboured both Stx1 and Stx2c prophages and it was observed in these strains that one Stx prophage was induced at a higher titre over the other. Genomic analysis predicted for the first time that the Stx2c phages package their DNA via cohesive ends. Further interrogation of the genomes also showed that two translational frameshift events, in different genes, are required for tail assembly and extension of host range respectively. In addition, each of the Stx2c prophages studied carried an anti-repression operon that may counteract the repression of the Stx1 prophage in-*trans* which could be a possible explaination for the increase in the production of Stx. This could be the mechanism as to why STEC

strains that harbour a Stx2c prophage in conjunction with another Stx prophage, in particular the Stx2a prophage, appear to be more virulent than other combinations of Stx prophages.

This thesis also reports the first evidence for Stx phage-mediated horizontal transfer of the locus of enterocyte effacement (LEE) pathogenicity island, as well as the characterisation of this Stx prophage. The mechanism of LEE mobilisation was determined to be likely due to a combination of both generalised and specialised transduction, and the incorporation of the LEE in recipient strains is likely due to homologous recombination. In addition to the discovery that an Stx phage can mediate the mobilisation of the LEE, genomic characterisation of this Stx prophage also revealed that it has a number of phage encoded genes that are predicted to enhance its ability to infect other susceptible bacterial strains by evading bacterial toxin-anti toxin defences and infecting a broad host range.

Overall, the results presented in this thesis suggested that Stx phages contribute more to bacterial pathogenesis than just the conference of the *stx* genes and that there are other interactions between the host and the Stx phage, which have resulted in STEC O157 becoming a successful pathogen.