Escherichia coli bacteriophages as candidates for phage therapy and their involvement in membrane vesicle biogenesis

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Certificate of Authorship/Originality

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Abbreviations

Abbreviation	Meaning
3D-SIM	Three-dimensional structured illumination microscopy
ºC	Degree Celsius
aa	Amino acid/s
Abi	Abortive infection
ATP	Adenosine triphosphate
att	Attachment site
BLAST	Basic Local Alignment Search Tool
bp	Base pair/s
CDS	Coding sequence
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CsCl	Caesium chloride
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
EOP	Efficiency of plating
ESBL	Extended spectrum β-lactamase
ESCs	Extended spectrum cephalosporins
EU	Endotoxin unit
ExPEC	Extra intestinal pathogenic *E. coli*
FQR	Fluoroquinolone-resistance
g	Relative centrifugal force
G+C	Guanine + Cytosine
h	Hour/hours
ICTV	International Committee on Taxonomy of Viruses
Kb	Kilo base
Kda	Kilo dalton
Kv	Kilo volt
L	Litre
LAL	Limulus amebocyte lysate
LB	Lysogeny broth
LPS	Lipopolysaccharide
M	Molar
Mb	Mega base
MDR	Multi drug resistant
Min	Minute/minutes
µl	Microlitre
mL	Mililitre
MLST	Multi locus sequence typing
mM	Miliomolar
mRNA	Messanger Ribonucleic acid
MVs	Membrane vesicles
MWCO	Molecular weight cut-off value
NCBI	National Centre for Biotechnology information
ng	Nanogram
nm	Nanometer
nr	Non-reductant nucleotide
nt	Nucleotides

Abbreviation	Meaning
NTP	Nucleotide triphosphate
PB	Phage buffer
pfu	Plaque forming unit
R-M	Restriction modification
RNA	Ribonucleic acid
rpm	Revolutions per minute
SAR	Sequence anchored release
SDS	Sodium dodecyl sulphate
Sec	Second/s
ST	Sequence type
Syd1	vB_EcoS_Syd1
Syd6	vB_EcoP_Syd6
Syd8	vB_EcoP_Syd8
T4	Bacteriophage T4
T7	Bacteriophage T7
TA	Toxin-antitoxin
TEM	Transmission electron microscopy
tRNA	Transfer ribonucleic acid
US FDA	The United States Food and Drug Administration
UV	Ultraviolet
v	Version

Research outputs

Publications

Mandal P. K., Sim E. M., Mansfield D., Haggerty L., Venturini C., Iredell J. R., Whitchurch C. B., & Petty N. K., (2020) Three virulent bacteriophages to combat the multidrug resistant *Escherichia coli* clone ST131 (Manuscript in preparation).

Mandal P. K., Ballerin G., Petty N. K., & Whitchurch C. B., (2020) Membrane Vesicles biogenesis in *Escherichia coli* through Bacteriophage infection (Manuscript in preparation).

Mandal P. K., Sim E. M., Whitchurch C. B., & Petty N. K., (2020) Phage purification and endotoxin removal utilising Triton X-114 and ultrafiltration (Manuscript in preparation).

Conference proceedings

Mandal P. K., Ballerin G., Petty N. K., & Whitchurch C. B., (July 2019) **ORAL PRESENTATION**. Membrane vesicles (MVs) as an outcome of bacteriophage mediated bacterial lysis. *Australian Society of Microbiology Annual Scientific Meeting*, Adelaide, Australia.

Abstract

The emergence of multidrug-resistant (MDR) bacterial pathogens is one of the biggest threats to global health. The WHO has recently prioritized the carbapenem-resistant Gram-negative bacteria, including *Escherichia coli*, as critical pathogens for which new therapeutic treatments are urgently required. However, there have been no new antibiotics approved in the past few decades and there is a trend of rapid development of bacterial resistance against the available antibiotics. Thus, non-conventional approaches are essential to control such MDR bacteria and therapy-utilising bacteriophages currently experiencing revival as an effective strategy. Bacteriophages are bacterial viruses that infect and multiply in bacteria thereby lysing them. Bacteriophages can only be cultivated in a susceptible bacterial host and may contain potential contaminants from bacterial cell. Membrane vesicles (MVs) are spherical nanostructures produced by bacteria and are also observed within bacteriophage preparations. However, there is no direct evidence linking bacteriophage activity and MVs formation. This Thesis established a collection of three new bacteriophages Syd1, Syd6, and Syd8 infecting one of the high risks MDR *Escherichia coli* Sequence Type 131 (ST131) and determined the source of MVs within bacteriophage preparations.

The genomic analysis determined that Syd1, Syd6, and Syd8 each lacked genes encoding for lysogeny, bacterial virulence, or antibiotic resistance. Electron microscopy and comparative genomic analyses revealed that Syd1, Syd6, and Syd8 were both morphologically and genetically distinct from each other and other previously characterized ST131 infecting bacteriophages. These three bacteriophages each possessed a completely distinct host recognition gene from each other and other closely related bacteriophages, indicating that each bacteriophage targeted a different surface receptor. Host range analysis on a panel of *E. coli* strains showed that all three bacteriophages can infect multiple ST131 strains efficiently and had different host range infectivity patterns, suggesting them as appropriate candidates for therapy. Live-cell super-resolution microscopy of *E. coli* infected with bacteriophages T4 and T7 showed that during bacteriophage infection, bacterial cells lyse explosively and MVs were formed from shattered membrane fragments. Electron microscopy revealed the presence of different forms of MVs within phage lysates, consistent with MVs formation through phage mediated bacterial lysis. A bacteriophage purification method utilising

ultrafiltration and Triton X-114 phase separation was optimised to remove endotoxins and MVs from bacteriophage preparations. Validation of the method on a panel of *E. coli* bacteriophages including Syd1, Syd6, and Syd8 showed the possibility of up to 5-log reduction in endotoxin and MVs were not detected from electron microscopy of any of these purified bacteriophages.

Chapter overview

This Thesis is composed of six chapters. Chapter one provides a general introduction to the *Escherichia coli* specifically a high-risk strain *E. coli* ST131, bacteriophage specifically tailed phages, phage therapy and preparation and purification of phages. Chapter two describes the materials and methodologies used to achieve the aims of this thesis. Chapter three details the isolation, morphology, genomic and host range characterization of three distinct bacteriophages Syd1, Syd6 and Syd8, which can infect and kill *E. coli* ST131 strains. Chapter four explores the membrane vesicles (MVs) biogenesis in *E. coli* through virulent bacteriophage infection and demonstrates the source of MVs within phage preparation. Chapter five describes a phage purification method, which is proposed for separating MVs from phages and getting pure phages with minimal endotoxin level. Chapter six discuss the main conclusions of the studies presented in this thesis and outlines recommendations for future work.