



University of Technology, Sydney

**DNA on the move: Investigation
into two mobile genetic elements
in *Vibrio* species**

Rita Antoinette Rapa

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CERTIFICATE OF ORIGINAL AUTHORSHIP

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

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Date:

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Table of contents:

Acknowledgements:	ii
Table of contents:	iii
List of Figures:	xi
List of Tables:.....	xiv
List of publications:.....	xv
Abbreviations:.....	xvi
Abstract:	xviii
Chapter 1: Introduction	1
1.1: Preface	1
1.2: <i>Vibrio</i> species: An overview	1
1.2.1: The diverse roles of vibrios.....	2
1.2.1.1: Mutual symbiotic relationships	3
1.2.1.2: Pathogenic relationships.....	4
1.2.1.2.1: Human pathogens.....	5
1.2.1.2.2: Marine animal pathogens	9
1.3: Lateral gene transfer: a major mechanism for bacterial evolution.....	10
1.3.1: Transformation.....	12
1.3.2: Conjugation.....	12
1.3.3: Transduction.....	13
1.3.3.1: Lysogenic conversion.....	14
1.3.4: Integration/maintenance of DNA sequences.....	15
1.4: Mobile genetic elements: a source of DNA	17
1.4.1: Plasmids	17
1.4.2: Transposons.....	18

1.4.3: Bacteriophage	20
1.4.4: Genomic islands	23
1.4.4.1: Genomic islands and the evolution of pathogenic/pandemic <i>V. cholerae</i> ...	25
1.4.5: Integrative conjugative elements: the tip of the ICEberg.....	26
1.5: LGT: a major mechanism for bacterial evolution	27
1.5.1: <i>V. cholerae</i> : a paradigm for the importance of LGT in adaptation and evolution	28
1.6: The integron/gene cassette system	31
1.6.1: A big black box in our understanding of gene cassette-associated function(s) ..	35
1.7: Project aims	41
1.7.1: How does the integron/gene cassette system impact on cell physiology?	41
1.7.2: How does a novel genomic island impact on cell survival and overall fitness? ..	41
Chapter 2: Materials and Methods	43
2.1: Bacterial strains and growth conditions	43
2.2: List of primers	51
2.3: DNA methods.....	54
2.3.1: PCR and agarose gel electrophoresis	54
2.3.2: Genomic DNA extraction	55
2.3.2.1: Crude extraction for PCR.....	55
2.3.2.2: XS buffer method	56
2.3.3: Plasmid extraction.....	56
2.3.4: Fosmid library construction, transposon mutagenesis and screening of RME ...	57
2.3.5: DNA and whole genome sequencing.....	58
2.3.6: Extraction, purification and quantification of DNA samples from agarose gels	58
2.3.7: Preparation of competent cells	58

2.3.8: Transformation of competent cells	58
2.3.8.1: Chitin induced transformation of non-O1/O139 <i>V. cholerae</i> strains with RME	59
2.3.9: Conjugation of integron gene cassettes from <i>E. coli</i> into <i>Vibrio</i>	59
2.3.9.1: Tri-parental conjugation of DAT722 deletion mutants	59
2.3.9.2: Bi-parental conjugation of DAT722 deletion mutants	60
2.3.10: Cloning of genes	61
2.3.10.1: Cloning of integron gene cassettes into pJAK16	61
2.3.10.2: Cloning of integron gene cassettes into pSU-pBAD	62
2.3.10.3: Cloning of <i>recA</i> _{S22} and <i>gfp</i> into pOriVn ₇₀₀	64
2.4: Construction of integron gene cassette deletion mutants in <i>V. rotiferianus</i> DAT722	64
2.5: Protein methods	65
2.5.1: Preparation of bacterial cells for 2D-PAGE analysis	65
2.5.1.1: Preparation of bacterial cells grown in LB20	65
2.5.1.2: Preparation of bacterial cells grown in 2M + glucose	65
2.5.2: Preparation of protein from bacterial cells for 2D-PAGE and secretome analysis	66
2.5.2.1: Removal of DNA and cell wall material from whole cell protein samples	66
2.5.2.2: Removal of salts and other small contaminants from whole cell protein samples	67
2.5.2.3: Supernatant protein extraction and gel electrophoresis	67
2.5.3: Quantification of protein in samples	68
2.5.3.1: Preparation of a protein standard for use in quantification	68
2.5.3.2: Quantification of total protein by densitometry	69
2.5.3.3: Fixing, staining and imaging of protein gels	70
2.5.3.4: Quantification of samples by densitometry	70

2.5.4: 2D-PAGE	71
2.5.4.1: Isoelectric focusing – the 1st dimension of separation	71
2.5.4.1.1: Rehydration of the IPG strip	71
2.5.4.1.2 IEF–apparatus set-up and loading the strip	72
2.5.4.2: Polyacrylamide gel electrophoresis–the 2nd dimension of separation	73
2.5.4.2.1: IPG strip equilibration.....	73
2.5.4.1.2: Polyacrylamide gel electrophoresis–apparatus set-up and loading the strip	73
2.5.5: Identification of differentially expressed or shifted proteins between wt DAT722 and the d16-60 mutant	75
2.5.5.1: PDQuest	75
2.5.5.2: Excision and trypsin digestion of protein spots	76
2.5.5.3. Liquid chromatography tandem mass spectrometry (LC-MS/MS).....	76
2.5.5.4. Identification of protein spots by comparing generated peptides to predicted proteins from the annotated genome sequence of <i>Vibrio rotiferianus</i> DAT722	77
2.6: Polysaccharide methods	78
2.6.1: Extraction and purification of loosely attached surface polysaccharide	78
2.6.2: Extraction of whole cell polysaccharide	78
2.6.3: Gel electrophoresis of whole cell and loosely attached polysaccharides	79
2.6.4: Silver staining of polysaccharide 1D gels	79
2.6.5: Congo red binding assays.....	80
2.6.5.1: Congo red colony morphology.....	80
2.6.5.2: Congo red liquid binding assays	80
2.6.6: Nuclear magnetic resonance of whole cell polysaccharide	80
2.7: Microscopy.....	81
2.7.1. Preparation of cells for fluorescence microscopy	81

2.7.2: Phase-contrast and fluorescence microscopy	81
2.7.3: Microscopy of congo red stained colonies.....	81
2.7.4: Inverted microscopy of crystal violet stained biofilms	82
2.8: Flow cytometry	82
2.8.1: Calcofluor staining of surface polysaccharide	82
2.8.2: Concanvalin A staining of surface polysaccharide	82
2.8.3: Performing flow cytometry.....	82
2.9: Biofilm assays	83
2.10: Bioinformatic analysis	83
2.10.1: Phylogenetic tree construction	84
2.11: Ultraviolet-light irradiation assays.....	84
2.12: Environmental stress assays.....	85
2.12.1: Oxidative stress assays.....	85
2.12.2: Iron depletion stress	85
2.12.3: Cold shock assays	85
2.13: Antibiotic assays	86
2.13.1: Minimum inhibitory concentration (MIC) assays.....	86
2.13.2: Mutation frequency assays	86
2.14: <i>recA</i> targeting experiments	86
Chapter 3: Investigating the role of the integron/gene cassette system on <i>Vibrio</i> physiology	88
3.1: Introduction.....	88
3.2: Results.....	89
3.2.1: Do gene cassette deletions influence growth in <i>V. rotiferianus</i> DAT722?.....	89
3.2.2: Gene cassette deletions do not affect environmental stress survival	92

3.2.3: Do gene cassette deletions affect regulation and post-translational modifications of whole cell and secreted proteins?	94
3.2.3.1: How does deletion of gene cassettes affect whole cell proteome?	94
3.2.3.2: An extracellular contaminating substance produced by d16-60 interferes with the IEF step in 2D-PAGE and gives insight into how gene cassettes affect DAT722 physiology.....	100
3.2.3.3: Does deletion of gene cassettes impact on the secretome?	101
3.2.3.4: Are gene cassettes involved in modification of glycosylated proteins?	102
3.2.4: Investigation into whether gene cassette deletions modify surface polysaccharide.....	104
3.2.4.1: Flow cytometry of cells stained with calcofluor and fluorescently labelled lectin	105
3.2.4.2: Gel electrophoresis of extracellular and lipopolysaccharides reveals potential minor changes in polysaccharide structures between wt DAT722 and deletion mutant d16-60.....	109
3.2.4.3: Congo red colony staining	112
3.2.4.4: Proton nuclear magnetic resonance spectroscopy of whole cell polysaccharides	113
3.2.5: A gene cassette deletion impacts on biofilm formation.....	117
3.3: Discussion	119
3.3.1: Gene cassette-associated products influence whole cell polysaccharide	119
3.3.2: Further insight into the effect of a gene cassette deletion on cellular physiology from the 2D-PAGE analysis.....	123
3.4: Future directions and conclusions	125
Chapter 4: Expression of foreign integron-associated gene cassettes: the identification of a novel gene cassette phenotype	128
4.1: Introduction.....	128
4.2: Results	129
4.2.1: Bioinformatic analysis of three gene cassettes.....	129

4.2.2: Cloning of gene cassettes into an IPTG-inducible expression vector results in amino acid substitution within gene cassette-encoded proteins.....	133
4.2.3: Does cloning gene cassettes downstream of an arabinose-inducible promoter avoid point mutations?	135
4.2.4: Does expression of gene cassettes affect growth?	140
4.2.5: Expression of <i>V. rotiferianus</i> DAT722 gene cassettes in <i>V. cholerae</i> S24.....	143
4.2.5.1: Do non-native gene cassettes affect growth of <i>V. cholerae</i> S24?	143
4.2.5.2: Expression of cassette 31 causes cell aggregation in <i>V. cholerae</i> S24	148
4.2.5.3: Expression of gene cassettes affects congo red binding capacity of <i>V. cholerae</i> S24 cells	150
4.2.5.4: Bacterial cell aggregation alters biofilm formation on hydrophilic and hydrophobic surfaces.....	152
4.3: Discussion	155
4.3.1: Plasmid expression systems in vibrios	155
4.3.2: New insight into how gene cassette-associated products influence bacterial cell physiology and virulence	156
4.3.2.1: How might gene cassette-induced cell aggregation influence cholera outbreaks?.....	157
4.4: Future directions and conclusions	158
Chapter 5: Characterisation and function of a genomic island inserted into the chromosome of non-O1/O139 environmental <i>Vibrio cholerae</i> strain S24.....	159
5.1: Introduction.....	159
5.2: Results.....	162
5.2.1: Identification of a novel genomic island in <i>V. cholerae</i> S24 containing <i>recA</i> and other DNA repair genes.....	162
5.2.2: Phylogenetic analysis of <i>recA</i> on genomic island proves it divergent from <i>recA</i> _{S24}	167
5.2.3: The <i>recA</i> genomic island circularises and is excised from the S24 genome	169
5.2.4: The <i>recA</i> genomic island targets the <i>recA</i> gene.....	171

5.2.5: The <i>recA</i> genomic island provides protection against ultraviolet irradiation in <i>E. coli</i>	175
5.2.6: The <i>recA</i> genomic island provides <i>E. coli</i> with increased protection against antibiotics.....	180
5.2.7: The <i>recA</i> genomic island increases spontaneous mutation frequency in <i>E. coli</i> when grown on media containing antibiotics.....	181
5.3: Discussion.....	184
5.3.1: The RME genomic island carries genes involved in DNA repair that are functional in <i>E. coli</i> and protect against induced DNA damage.....	184
5.3.2: Are there implications of antibiotic protection due to RME?.....	187
5.4: Future directions and conclusions.....	189
Chapter 6: General Discussion.....	191
6.1: The function of integron-associated gene cassettes in <i>Vibrio</i> species: the tip of the iceberg revealed.....	191
6.2: A genomic island in an environmental strain of <i>V. cholerae</i> highlights the importance of the ‘environment’ in driving adaptation of bacteria.....	194
6.3: Overall conclusions.....	196
References:.....	198
Appendix:.....	215

List of Figures:

Figure 1.1: Timeline detailing the emergence of new cholera pandemics.....	7
Figure 1.2: Pictorial description of the lifestyles of <i>V. cholerae</i>	8
Figure 1.3: Mechanisms of LGT.....	11
Figure 1.4: The lytic and lysogenic lifecycles of bacteriophage.....	15
Figure 1.5: Composite transposon.....	20
Figure 1.6: General features of genomic islands.....	23
Figure 1.7: Importance of MGEs in the emergence of <i>V. cholerae</i> virulence.....	30
Figure 1.8: The structure of the integron/gene cassette system.....	32
Figure 2.1: pJAK16 map.....	62
Figure 2.2: pSU-pBAD map.....	63
Figure 2.3: Quantification of protein samples by densitometry.....	71
Figure 2.4: Representative image of a gel derived from 2D-PAGE of whole cell proteins from <i>Vibrio rotiferianus</i> DAT722.....	75
Figure 3.1: Growth of wt DAT722 and isogenic deletion mutants.....	91
Figure 3.2: Environmental stress assays.....	94
Figure 3.3: Genomic localisation of unknown protein.....	99
Figure 3.4: Contaminating substance affecting IEF of 2D-PAGE analysis.....	100
Figure 3.5: Gel electrophoresis of supernatant proteins.....	101
Figure 3.6: Glycoprotein gel electrophoresis.....	103
Figure 3.7: Overview of cell envelope.....	105
Figure 3.8: Auto-fluorescence in <i>V. rotiferianus</i> DAT722.....	107
Figure 3.9: Concanavalin A lectin binding of <i>V. rotiferianus</i> DAT722 and deletion mutant cells.....	109
Figure 3.10: Fluorescence microscopy of wt DAT722 cells stained with concanavalin A.....	109

Figure 3.11: Gel electrophoresis of total surface polysaccharides.	111
Figure 3.12: Congo red colony morphology.	113
Figure 3.13: ¹ H NMR spectra.	115
Figure 3.14: ¹ H NMR replicate spectra.	116
Figure 3.15: Graph showing optical density of crystal violet stained cells.	117
Figure 3.16: Microscopy of crystal violet stained cells.	118
Figure 3.17: Proposed mechanism for the production of surface polysaccharide diversity through deletions, rearrangements and insertions in the cassette array.	122
Figure 4.1: Putative identification for protein encoded by VSD31.	130
Figure 4.2: Putative identification for protein encoded by VSD54.	131
Figure 4.3: Putative identification for protein encoded by VSD78.	132
Figure 4.4: Translated protein sequence of cassette 31 shows an amino acid substitution.	134
Figure 4.5: Translated protein sequence of cassette 78 shows an amino acid substitution.	135
Figure 4.6: Protein sequence encoded by cloned cassette 31 without any amino acid substitution.	137
Figure 4.7: Protein sequence encoded by cloned cassette 54 without any amino acid substitution.	138
Figure 4.8: Protein sequence encoded by cloned cassette 78 without any amino acid substitution.	139
Figure 4.9: Growth curve of deletion mutants with pSU-pBAD in LB20.	141
Figure 4.10: Growth curve of deletion mutants with pSU-pBAD in 2M+glucose.	142
Figure 4.11: Effect of VSD31, VSD78 and VSD54 on growth of <i>V. cholerae</i> S24 in LB5.	146
Figure 4.12: Effect of VSD31, VSD78 and VSD54 on growth of <i>V. cholerae</i> S24 in 2M + glucose.	148
Figure 4.13: Phase contrast microscopy of cell aggregation due to expression of VSD31.	149
Figure 4.14: Congo red liquid culture binding.	151

Figure 4.15: Biofilms grown on a hydrophilic surface.	153
Figure 4.16: Biofilms grown on a hydrophobic surface.	154
Figure 5.1: Gel electrophoresis of product generated from <i>recA</i> _{S24} PCR.	163
Figure 5.2: Pictorial representation of how contig gaps containing the genomic island were closed and gel electrophoresis of PCR products generated.	164
Figure 5.3: Genes carried by the <i>recA</i> mobile element and genetic context within <i>V. cholerae</i> S24.	166
Figure 5.4: Phylogenetic tree of 30 <i>recA</i> nucleotide sequences from the <i>Vibrio</i> genus.	169
Figure 5.5: RME can circularise from the S24 chromosome and leaves behind an intact copy of <i>recA</i> _{S24}	170
Figure 5.6: RME insertion into and excision from the S24 host genome.	171
Figure 5.7: The <i>recA</i> genomic island targets <i>recA</i>	173
Figure 5.8: Possible insertion events of RME into <i>recA</i> from <i>V. cholerae</i> S22.	175
Figure 5.9: Growth of fosmid constructs in EPI300 in LB5 medium.	178
Figure 5.10: Ultraviolet-irradiation assays of <i>E. coli</i> containing <i>recA</i> genomic island.	179

List of Tables:

Table 1.1: Representative list of vibrios that hold symbiotic relationships with aquatic higher organisms	4
Table 1.2: Representative list of vibrios that infect human and marine animals	5
Table 1.3: Mechanisms for DNA transfer and maintenance in the bacterial cell ¹	17
Table 1.4: A short list of plasmids present in vibrios ¹	18
Table 1.5: Short representative list of vibriophages identified ¹	22
Table 1.6: Examples of genomic islands and their encoded functions	24
Table 1.7: A short list of bacterial species that contain chromosomal integrons	34
Table 1.8: Brief list of some non-antibiotic resistance functional ORFs in gene cassettes ..	39
Table 2.1: Media and solution constituents	45
Table 2.2: 2M + 0.2% glucose constituents	47
Table 2.3: List of bacterial strains and plasmids	48
Table 2.4: List of primers	51
Table 2.5: Silver staining protocol	79
Table 2.6: Flow cytometry parameters	83
Table 3.1: Differentially expressed spots between deletion mutant d16-60 and wt <i>V. rotiferianus</i> DAT722 in LB20	97
Table 3.2: Differentially expressed spots between deletion mutant d16-60 and wt <i>V. rotiferianus</i> DAT722 in 2M + glucose	98
Table 3.3: Putative genes in <i>V. rotiferianus</i> DAT722 involved in glycosylation of proteins	102
Table 5.1: Minimal inhibitory concentration (MICs*)	181
Table 5.2: Rifampicin ^a mutation frequencies	182
Table 5.3: Nalidixic acid ^b mutation frequencies	183

List of publications:

Rita A. Rapa, Christopher Allen, Maurizio Labbate. 2014. Expression of gene cassettes affect polysaccharide phenotypes. (Manuscript in preparation).

Rita A. Rapa, Atiqul Islam, Leigh G. Monahan, Ankur Mutreja, Nicholas Thomson, Ian G. Charles, H. W. Stokes, Maurizio Labbate. 2014. A genomic island integrated into *recA* of *Vibrio cholerae* contains a divergent *recA* and provides multi-pathway protection from DNA damage. *Environmental Microbiology*. (Appendix 3)

Rita A. Rapa and Maurizio Labbate. 2013. The function of integron-associated gene cassettes in *Vibrio* species: the tip of the iceberg. *Frontiers in Microbiology*. 4:385 (Appendix 5)

Rita A. Rapa, Ronald Shimmon, Steven P. Djordjevic, H.W. Stokes, Maurizio Labbate. 2013. Deletion of Integron-associated gene cassettes impact on the surface properties of *Vibrio rotiferianus* DAT722. *PLoS ONE*. 8:e58430 (Appendix 2)

Abbreviations:

LGT	lateral gene transfer
Indels	insertions and deletions
MGE(s)	mobile genetic element(s)
spp.	species (plural)
sp.	species (singular)
μ	micro; 10 ⁻⁶
m	metre
cm	centimetre
%	percent
mL	millilitre
L	litre
DNA	deoxyribonucleic acid
Kb	kilobases
bp	base pairs
kbp	kilo base pair
IS	insertion sequence
IR	inverted repeat
VPI	<i>Vibrio</i> Pathogenicity Island
VSP	<i>Vibrio</i> Seveth Pandemic
DR	direct repeat
ICE	integrative conjugative element
ORF	open reading frame
OD	optical density

hr	hours(s)
s	second(s)
ms	millisecond(s)
wt	wild-type
2D-PAGE	two-dimensional polyacrylamide gel electrophoresis
1D-PAGE	one-dimensional polyacrylamide gel electrophoresis
LC-MS/MS	liquid chromatography tandem mass spectrometry
kDa	kilo-dalton
LPS	lipopolysaccharide
CPS	capsular polysaccharide
EPS	extracellular polysaccharide
NMR	nuclear magnetic resonance
OMP	outer membrane protein/porin
VSD	<i>Vibrio</i> species DAT722
IPTG	isopropyl β -D-1-thiogalactopyranoside
V	volts
PCR	polymerase chain reaction
M	molar
UV	ultra violet
GI	genomic island

Abstract:

Vibrios are a group of Gram negative rod-shaped bacteria that are ubiquitous in marine and estuarine environments. They exist as both free-living organisms and in association with a variety of hosts such as humans, coral, marine animals and plants. *Vibrio cholerae* is the most notorious of vibrios, being the causative agent of the devastating intestinal disease cholera in humans.

Lateral gene transfer (LGT), a process that allows DNA transfer between bacterial cells, has largely driven the rapid evolution in *V. cholerae* and other *Vibrio* species. In some strains of *Vibrio* species at least 20% of genomic content has arisen from LGT events. With respect to *V. cholerae*, the two most important virulence factors: cholera toxin encoded by the *ctxAB* genes and intestinal adhesion encoded on the vibrio pathogenicity island (VPI-1) have been acquired *via* mobile genetic elements transferred by LGT. Thus, these two virulence factors convert toxigenic *V. cholerae* into a paradigm for the importance of LGT, demonstrating how seemingly avirulent strains of *V. cholerae* become capable of causing epidemic/pandemic outbreaks (Uma *et al.*, 2003).

Mobile genetic elements include but are not exclusive to: transposons, integrons, conjugative elements and genomic islands. Research performed in this thesis is focussed on the study of the integron and a genomic island and how phenotypes they confer contribute to the adaptation of two *Vibrio* species: *V. rotiferianus* and *V. cholerae*.

Briefly, integrons are a two-component genetic recombination system present in the chromosome of almost all *Vibrio* species. The integron incorporates mobile genes termed gene cassettes into a reserved genetic site *via* site-specific recombination, named the integron/gene cassette system. The integron consists of three basic elements: an integrase gene (*intI*), an attachment site (*attI*) and a promoter (P_c). Gene cassettes generally contain a single open reading frame (ORF) and an IntI-identifiable recombination site called *attC*. Insertion (and excision) of gene cassettes is driven by an integrase-mediated recombination between *attI* and *attC*. Multiple insertion events lead to the accumulation of cassettes to form a cassette array. In vibrios, cassette arrays are uniquely large, sometimes containing hundreds of cassettes that make up a 1-3% of the entire genome. There is a consensus that

these gene cassettes add to the adaptive potential of vibrios and have likely been an important driver in the evolution of vibrios into their respective niches. How this is achieved has been difficult to understand given that 80% of gene cassettes are novel and consequently of unknown physiological function. Using a number of chemical, proteomic and molecular techniques this thesis has shown that gene cassette(s) present in the chromosome of a model *Vibrio* organism (*V. rotiferianus* DAT722) are altering bacterial surface properties. Changes to bacterial surface properties can be important in bacterial-host interactions importantly; biofilm formation, protozoan grazing and pathogen-host association. This thesis also examines how another mobile genetic element; a novel genomic island, aids in the repair of damaged DNA in *V. cholerae*, giving the organism an advantage in both the environment and in the disease causing state within humans. Our knowledge of how LGT has and continues to drive bacterial adaptation and evolution has only uncovered the tip of the iceberg.