University of Technology, Sydney

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

Rita Antoinette Rapa

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy: Science

2014

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.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student:

Date:

Acknowledgements:

The undertaking of my PhD has been one of the most challenging but rewarding experiences so far. Although at times completing my doctorate has felt like a lonely and isolating journey, there are many people who have supported me along the way and I simply could not have gotten to the end without them.

Firstly, I would like to acknowledge the unwavering support and guidance of my two supervisors Dr Maurizio Labbate and Dr Hatch Stokes. I simply could not have achieved the academic fulfilment I have without you both. I thank you both for your mentorship, assistance with publications and for the many hours of proofreading this thesis. Maurizio, a massive thank you for your ongoing help in the lab, teaching me everything you know and for your friendship over the past 4 ½ years. I will dearly miss our laughs and inside jokes; I expect regular email updates! Hatch, thank you for your kindness and ongoing support with my PhD and career, even after your retirement. To my co-supervisor Professor Ian Charles, thank you for your academic guidance, financial support, philosophical conversations and the 'tea and buns.'

A huge thank you to past members of the Stokes Lab, particularly Elena Martinez, Natasha Vanegas and Piklu Roy Chowdhury, you have made my PhD a happy and exciting period of my life. To my fellow PhD students: Alex Gale, Raquel Alvarado, Ben Raymond, Sam Burns, Amelia Hynen, Andrew Liew, Erin Gloag, Shirin Ansari, Chris Allen, Jessica Tout and Lauren Messer. Thank you all for putting up with my tears, whinging and incessant snorts, accompanied by random outbursts of song and dance over the years. You have all touched my life in so many ways and I am going to miss seeing every one of you on a daily basis.

To Leigh Monahan, I cannot thank you enough for all your support, praise, reassurance and encouragement over the course of my PhD. You have helped me accomplish every task I have set out to do and I know this would not have been possible without you.

To my family: Mum, Papa, Loretta, David, AnneMarie and Nicko. You have all offered words of support throughout my academic life. Thank you for all you do and most importantly for trying to appear enthused when I email you my publications to read. Lastly, I would like to acknowledge the financial assistance of the Faculty of Science, University of Technology, Sydney in providing me with a scholarship for 3 ½ years.

Table of contents:

List of Figures:

List of Tables:

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	*Vibrio* Pathogenicity Island
VSP	*Vibrio* 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	*Vibrio* 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.