# Investigating the role of the *V. cholerae* integron/gene cassette system in biofilm formation and resistance to protozoa

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# CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Md Hafizur Rahman declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Sciences, Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

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# Abstract

*Vibrio cholerae* is a marine aquatic bacterium and the causal agent of the devastating diarrhoeal cholera disease that sickens millions of people each year. Persistence in the environment of this pathogen is an important area of research as this is where is resides in between periodical disease outbreaks. In the environment, *V. cholerae* deals with numerous stresses including temperature fluctuations, lack of nutrients, salinity and bacteriophage. Additionally, predatory action by free-living heterotrophic protists called protozoa is a major problem for *V. cholerae* growth and survival in the environment. Several mechanisms are employed by *V. cholerae* in environmental persistence, of which biofilm formation is considered a key mechanism. *V. cholerae* preferably forms biofilm on chitin, a long chain polymer present in the exoskeletons of crustaceans in the aquatic environment which apart from being a nutritive source, induces natural competency that allows free uptake of DNA through a mechanism of lateral gene transfer (LGT) called transformation. LGT is when DNA is transferred between bacterial cells and is considered an important evolutionary mechanism in *V. cholerae* through acquisition of novel genetic traits usually on mobile genetic elements such as genomic islands, phage or transposons. Chromosome 2 of *V. cholerae* carries one such mobile genetic element called the integron that facilitates the insertion of mobile genes called gene cassettes and contains more than 150 gene cassettes of which 80-90 % are of unknown or uncharacterised function. Integration, deletion or rearrangement of gene cassettes is dependent on a recombinase called the integron-integrase (encoded by *intIA*) that recognises *attC* sites associated with gene cassettes and facilitates their insertion within the *attI* site of the integron and to a lesser extent, *attC* sites of gene cassettes in the cassette array. *intIA* is induced by the SOS response a regulatory cascade requiring RecA that is activated by the presence of single-stranded DNA (ssDNA) due to stalled DNA replication from damage (e.g. fluoroquinolones, β lactams, UV exposure) or from acquisition of ssDNA from LGT processes.

In order to study chitin induced transformation and *intIA* transcription in *V. cholerae*, this thesis described the construction of circular and linear gene cassettes and investigated their transfer into the *V. cholerae* chromosomal integron of chitin-competent cells and demonstrating that the integron is a novel site for adding DNA for complementation of mutations in *V. cholerae*. Additionally, insertion of the artificial gene cassettes into *attI* and two different *attC* sites were shown to affect bacterial surface properties and biofilm formation most likely due to enhanced transcription of downstream gene cassettes due to the presence of internal promoters in the

artificial gene cassettes. Finally, one of the artificial gene cassettes was used to investigate cassette transfer dynamics in *V. cholerae* in the presence of two bacteriovorist protozoa, the ciliate *Tetrahymena pyriformis* and the amoeba *Acanthamoeba castellanii*. This thesis shows that following internalization and packaging of *V. cholerae* into the food vacuoles (also called phagosomes) of both protozoa, intracellular ROS induces the SOS response leading to enhanced integron-integrase expression and gene cassette recombination (up to 405-fold more cassette integration). ROS production is a key feature of killing within the phagosome causing DNA damage leading to mutagenesis that results in stalling of DNA replication and generating an excess of single ssDNA. In addition, this thesis shows that due to the indiscriminate feeding behaviour of protozoa, co-localisation of different species such as *V. cholerae* and *E. coli* in the same phagosome can facilitate LGT. It is shown that *V. cholerae* utilizes its T6SS to kill and release artificial gene cassette DNA from *E. coli* to make it accessible for uptake and subsequent integron-integrase mediated integration.

Taken together, this thesis, through the creation of artificial gene cassettes to study integron integration dynamics, highlights the importance of integron-associated gene cassettes in biofilm formation and shows the importance of protozoa in driving LGT-driven adaptation and evolution in *V. cholerae*.

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# List of Abbreviations

| | |
|---|---|
| AMP | Antimicrobial peptide |
| ANOVA | Analysis of variance |
| ATCC | American type culture collection |
| ATR | Acid tolerance response |
| C$^{o}$ | Degrees celcius |
| cAMP | Cyclic adenosine monophosphate |
| CAI | Cholera autoinducer |
| c-di-GMP | Cyclic di-guanosine monophosphate |
| CFU | Colony forming unit |
| CLSM | Confocal laser scanning microscopy |
| CT | Cholera toxin |
| DGC | Diguanylate cyclase |
| DR | Direct repeat |
| dsDNA | Double stranded deoxyribonucleic acid |
| eDNA | Extracellular DNA |
| EFV | Expelled food vacuole |
| x *g* | Gravitational force |
| GI | Genomic island |
| GFP | Green fluorescent protein |
| h | Hour |
| HOCl | Hypochlorous acid |
| ICE | Integrative conjugative element |
| IS | Insertion sequence |
| IR | Inverted repeat sequence |
| LB | Luria Bertani broth |
| LPS | Lipopolysaccharide |
| LCV | *Legionella*-containing vacuole |
| LGT | Lateral gene transfer |
| MGE | Mobile genetic element |
| min | Minute |
| ml | Millilitre |

| | |
|---|---|
| µl | Microlitres |
| mm | Millimetre |
| mM | Millimolar |
| µM | Micromolar |
| MSHA | Mannose-sensitive haemagglutinin |
| nM | Nanomolar |
| NSS | Nine salts solution |
| NaCl | Sodium chloride |
| OD | Optical density |
| ORF | Open reading frame |
| PCR | Polymerase chain reaction |
| PDE | Phosphodiesterase |
| PYG | Proteose yeast extract |
| QS | Quorum sensing |
| rpm | Revolutions per minute |
| ROS | Reactive oxygen species |
| RNS | Reactive nitrogen species |
| RT | Room temperature |
| RER | Rough endoplasmic reticulum |
| ssDNA | Single stranded deoxyribonucleic acid |
| T6SS | Type VI secretion system |
| TCP | Toxin-coregulated pili |
| VPI-1 | Vibrio pathogenicity island-1 |
| VPI-2 | Vibrio pathogenicity island-2 |
| VPS | Vibrio polysaccharide |
| WHO | World Health Organization |
| WT | Wild type |