# The pleiotropic effects of a *tolC* mutation in *Vibrio furnissii*

Submitted by

**Hannah Louise Upton Tape**

to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences

(September 2012)



I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Hannah Tape

## ABSTRACT

The ability of bacteria to successfully adapt to changing environments allows for increased resistance to antibiotics, an increasing problem in drug development. Efflux via the cellular membrane is one of the major and most significant mechanisms bacteria employ for defense against antimicrobials. The outer membrane protein, TolC, is a nonspecific channel with broad substrate specificity and is a fundamental constituent of a number of multidrug resistance (MDR) efflux systems. TolC, which interacts with membrane bound antiporters to export various substrates, including antimicrobials, has been a focus for antibiotic development. The capacity to expel a wide range of compounds such as toxins, bile, dyes and detergents, further establishes the significance of these systems in aiding bacterial survival. Continued investigation and discovery of compounds secreted via TolC is required to fully understand the role of efflux systems.

In addition to exporting compounds out of cells, TolC has been researched in connection with a number of metabolic processes including virulence, motility and quorum sensing. This work contributes to knowledge of interactions between these pathways by analysing phenotypic changes and alterations in gene expression in the *tolC*$^{-}$ mutant within *Vibrio furnissii. Vibrio* species are strongly associated with a number of reported phenotypic changes induced by mutations in *tolC,* particularly virulence and quorum sensing; the emerging pathogen *V. furnissii* is therefore a model organism for this study. Notably, this bacterium has become significant within the bio-fuel industry due to reports that it is able to synthesise large quantities of hydrocarbons. The potential for bacteria to produce hydrocarbons and fatty acids suitable for use in bio-fuels has considerable industrial applications. This work establishes the quantities of hydrocarbons and lipids present in *V. furnissii* and examines the role of TolC in the secretion of these compounds.

Resistance nodulation division efflux pumps are a class of MDR systems that have been identified as targets for antimicrobial drug development. Understanding the consequence of disrupting the integral outer membrane component TolC, is therefore of significant interest. This work examines the connection between pathways disrupted within *tolC*$^{-}$ mutants, showing that quorum sensing gene regulation is altered within the mutant and subsequently, expression of the virulence factor AphB is increased. Reduced motility is also observed in the *tolC*$^{-}$ mutant, a phenotype that appears to result from disruption of the membrane and mis-assembled flagella. Investigation into cellular stress, recently reported to occur within *tolC*$^{-}$ mutants, was performed by comparing the capability of wild type and mutant cells to metabolise selenite, and cope with the subsequent increase in superoxides generated. Results show that *tolC*$^{-}$ mutants employ mechanisms which cope with the inevitable intracellular build up of substrates, particularly those that may be toxic to the cell.

## ACKNOWLEDGMENTS

I would like to thank Prof. John Love and Prof. Clive Butler for all their support and encouragement throughout this PhD and my scientific career to date. Thanks to Prof. Rob Lee and co-workers at Shell Global Solutions for the opportunity and assistance throughout this project. I am very grateful to Prof. Laura Piddock at the University of Birmingham and Prof. Paul Williams at the University of Nottingham for their help and advice during this work.

Further thanks to Dr. Mark Wood for the synthesis of compounds required in this study and Dr. Hannah Florence for assistance with LC-MS. I would also like to thank Dr. David Parker for GC-MS analysis of samples and staff at the Nottingham Arabidopsis Stock Centre for preparation and hybridization of Microarray Chips. Thank you to Dr. Thomas Lux and Dr. Christine Sambles for designing the Affymetrix Genechips and for guidance with bioinformatics and transcriptome analysis. Further thanks to Peter Splatt for preparation of cells for transmission electron microscopy and for assistance with confocal microscopy. I wish to thank Dr. John Dowdle for providing the *tolC*$^{-}$ mutant studied in this work and Richard Tennant for assitance with FACS.

Thanks to Dr. Elizabeth Dridge for her advice and friendship throughout my time studying within the department and colleagues in the Mezzanine office and the Biocatalyis Centre who have always been on hand for a scientific chat or an after work drink. Special thanks to my family, especially my Mum for her ongoing support and to my friends and partner Tom who, as always, have been a huge encouragement.

TABLE OF CONTENTS