

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

Submitted by

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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.

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