Exploring the evolvability of resistance determinants in bacteria

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ABSTRACT

Antibiotic resistance poses a major risk to human and animal health. It has impacted on morbidity and mortality rates and has increased hospitalisation costs worldwide. To stand a fighting chance in this losing battle, it is important to understand the biochemical and evolutionary pathways that give rise to resistance. The goals of this thesis were to gain mechanistic insights into new resistance pathways and to explore whether protein functions associated with resistance could be evolved artificially.

In the first part of this thesis, I report that many pre-existing gene products in a non-pathogenic bacterium (*E. coli*) are able to impart resistance towards different classes of antibiotics. Investigating the relationship between fitness and resistance revealed that it was complex and as a result, it was difficult to predict how resistance would impact on bacterial fitness. It is apparent that the reservoir of resistance elements in non-pathogenic bacteria is much larger than previously appreciated, and may provide a rich source of resistance genes that could be co-opted by pathogenic bacteria.

In the second part of the thesis, specific examples were selected to explore the evolvability of resistance functions. Weak resistance activities of carbonic anhydrase and three proteins of unknown native function (YeaD, YdfW and YejG) could not be improved by directed evolution experiments in this study. Nonetheless, a novel multi-step route to high level tobramycin resistance involving YejG was discovered. Over-expression of YejG in the presence of a chromosomal mutation in the *fusA* gene (G1478T) endowed the bacterium with maximal resistance to tobramycin. In addition, a function of YejG in mediating an early entry into the log phase of growth has been uncovered.

Overall, the results in this thesis have revealed that there are even more pathways to resistance than previously appreciated. Weak resistance functions may not necessarily be easily evolvable, however, they may facilitate the emergence of mutations that confer higher levels of resistance. Ultimately, it is hoped that gaining a deeper insight into resistance pathways and how they evolve will help in the development of the new drugs that we so desperately need.

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Dedicated to my Father, Mr Alex Hanson-Manful, thank you for being my source of inspiration and for giving me the opportunity and investing in me all these years.

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Contents

List of illustrations

List of Tables

Abbreviations

Abbreviation	Description
ASKA	A complete Set of *Escherichia coli* K-12 Open Reading Frame Achive
CA	Carbonic anhydrase
Cfu	Colony-forming unit
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic acid
epPCR	Error-prone PCR
GFP	Green fluorescent protein
HGT	Horizontal gene transfer
His_6	Hexa-histidine
IPTG	β-D-1-thiogalactopyranoside
IS	Insertion sequences
lacZ	β-galactosidase
LB	Lysogeny broth
metC	Cystathionine β-lyase
MIC	Minimum Inhibitory Concentration
NoIns	pCA24N plasmid without any insert (negative control plasmid)
OD_{600}	Optical density measured at 600 nm
SE	Standard Error
SOC	Super Optimal Broth with Catabolite repression
Tris	Tris(hydroxymethyl)aminomethane
W	Relative fitness
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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