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Bacterial filamentation as a survival strategy: identification and characterisation of a novel cell division inhibitor in *Escherichia coli*

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Certificate of Authorship/ Originality

I, Shirin Ansari declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the i3 Institute, Faculty of Science at the University of Technology Sydney. This thesis is wholly my own work unless otherwise reference 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.

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Abstract

Bacterial cell division is tightly regulated to ensure that division occurs at the correct time and position in order to create two viable, genetically identical, daughter cells. In addition to correct timing and positioning, the inhibition of division is also important for survival in certain conditions. This inhibition of division results in filamentous cells, a process where cell growth and DNA replication continues in the absence of division, resulting in elongated cells. This is an important survival mechanism utilised by several bacteria in response to an environmental stimuli, including during pathogenesis and exposure to antibiotics. However, the underlying mechanisms of filamentation and regulators of cell division that enable this unique morphology remain largely unknown. A recent high-throughput over-expression screen in *Escherichia coli* identified several potential division inhibitors, including *ymfM*, a gene encoded within the e14 prophage. The overall aim of this thesis was to understand the biological condition in which YmfM may be functioning, as well as how it may be acting to inhibit division.

The initial aim of this thesis was to verify the genes from the original screen that are responsible for causing filamentation. From this the expression of *ymfM* was shown to cause a complete inhibition of cell division and became the primary focus of this work.

The e14 prophage is thought to encode for an SOS inducible cell division inhibitor, SfiC. However the exact gene responsible for this is unknown. In this thesis, YmfM was identified to be SfiC and is up-regulated by the SOS response. The inhibition of cell division during SOS has traditionally been attributed to SulA, which inhibits FtsZ polymerization and is activated by the RecA pathway. However, we have identified the

likely role of *ymfM* in inhibiting division during SOS, suggesting that alternative pathways exist during stress.

Bioinformatics analysis identified the context in which *ymfM* functions; it is conserved to *E. coli* and closely related gram-negative bacteria. Further, it was shown to be likely that *ymfM* functions with two other genes within the e14 prophage, *ymfL* and *oweE*. Finally, the initial characterisation of the mechanism of action of YmfM indicates that it inhibits division at the level of FtsZ ring assembly (early stages of division) and is independent of known cell division inhibitors SulA, MinC and SlmA.

While more work is needed to fully characterise YmfM, this work highlights that there are multiple pathways which may inhibit cell division during stress and raises the question of the beneficial role of prophage encoded inhibitors for bacterial survival. Having a greater understanding of filamentation will not only enable us to understand the intricacies of cell division inhibition, but also how bacteria are able to cope with stress.

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Publications and Conference Proceedings

Publications:

Mediati D, Burke C, **Ansari S**, Harry EJ, Duggin IG (2018) High-throughput sequencing of phenotypically enriched DNA libraries: discovering mediators of reversible bacterial filamentation, Under Review at BMC Genomics

Ansari, S (2016) Bacterial filamentation as a survival strategy. ASM Syntrophy, Volume 17 Issue 4 Page 3

Conferences proceedings:

1. **Ansari S**, Burke C, Duggin IG, Harry EJ (2014) Bacterial filamentation as a survival strategy: identification and characterisation of genes causing filamentation in *Escherichia coli,* **Australian Society for Microbiology Annual Scientific Conference, Melbourne, Poster Session**

2. **Ansari S**, Burke C, Bottomley A, Duggin IG, Harry EJ (2015) Bacterial filamentation as a survival strategy: identification and characterisation of genes causing filamentation in *Escherichia coli,* **BacPath 13: Molecular Analysis of Bacterial Pathogens, Melbourne, Poster Session**

3. **Ansari S**, Burke C, Bottomley A, Duggin IG, Harry EJ (2016) Bacterial filamentation as a survival strategy: identification and characterisation of genes causing filamentation in *Escherichia coli,* **Australian Society for Microbiology Annual Scientific Conference, Perth, Poster Session**

4. **Ansari S**, (2016) Bacterial filamentation as a survival strategy: identification and characterisation of genes causing filamentation in *Escherichia coli,* **East Coast Bacillus Meeting, Sydney, Oral Presentation**

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Abbreviations

aa	Amino acid
Ab	Antibody
ADP	Adenosine 5'-diphosphate
AGRF	Australian Research Genome Facility
ATP	Adenosine 5'-triphosphate
B.	Bacillus
BACTH	Bacterial two hybrid system
BP	Band pass
bp	Base pair(s)
BSA	Bovine serum albumin
°C	Degrees Celsius
CFU	Colony forming unit
Da	Dalton(s)
DAPI	4'6-diamidino-2-phenylindole
Δ	Deletion of gene
DNA	Deoxyribonucleic Acid
E.	*Escherichia*
ECL	Enhanced chemi-luminescence
EDTA	Ethylenediaminetetraacteic acid
et al.	and others
fts	Filamentation temperature sensitive
g	Centrifugal force
g	Gram(s)
gDNA	Genomic DNA
GDP	Guanosine diphosphate
GFP	Green fluorescent protein
GTP	Guanosine triphosphate
h	Hour(s)
IFM	Immunofluorescence microscopy

IgG	Immunoglobulin G
IPTG	isopropyl-1-thio-β-D-galactopyranoside
kb	Kilobase(s)
L	Litre(s)
m	Milli- (10^3)
M	Moles per litre
mg	milligram
min	Minute(s)
mL	Millilitre(s)
MQW/MilliQ	Milli-Q purified water
MSA	Mineral salts A
n	Nano- (10^3)
N/A	Not applicable
NEB	New England Biolabs
OD_{600}	Optical Density at 600nm
ORF	Open reading frame
PAGE	Polyacrylamide gel electrophoresis
PBP	Penicillin binding protein
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pH	Power of Hydrogen
RNA	Ribonucleic Acid
rpm	Revolutions per minute
S.D	Standard deviation
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
spp.	Species
TEV	Tobacco Etch Virus
Tris	Tris(hydroxymethyl)methylamine
ts	Temperature sensitive
UV	Ultraviolet
V	Volts(s)
v/v	Volume per volume

w/v	Weight per volume
μ	Micro- (10^6)