Bacterial filamentation as a survival strategy: a goldmine for the discovery of new cell division regulators

Samuel J. Burns

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University of Technology, Sydney

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Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the written preparation of the thesis, and all experimental work associated with it has been carried out solely by me, unless otherwise indicated.

Finally, I certify that all information sources and literature used are acknowledged in the text.

Samuel J. Burns, August 2016

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Abbreviations

aa	amino acid
AG	arabinogalactan
AGRF	Australian Genome Research Facility
ATc	anhydrotetracycline
ATP	Adenosine 5’ triphosphate
B.	*Bacillus*
BCG	Bacillus Calmette-Guérin
BLAST	basic local alignment search tool
bp	base pair(s)
BP	band pass
BSA	bovine serum albumin
°C	degrees Celsius
chrDNA	chromosomal DNA
cm	centimeters
CCD	charged coupled device
DAPI	4'6-diamidino-2-phenylindole
dATP	deoxyadenosine 5’-triphosphate
dCTP	deoxycytidine 5’-triphosphate
dGTP	deoxyguanosine 5’-triphosphate
DNA	deoxyribonucleic acid

dTTP	deoxythymidine 5'-triphosphate
E.	*Escherichia*
EM	electron microscopy
EsC	environmental sub-clone
et al.	and others
FSC	forward scatter
fts	filamentation temperature sensitive
g	centrifugal force
g	gram(s)
gDNA	genomic DNA
GFP	green fluorescent protein
h	hour(s)
kb	kilo base pair(s) (1000 bp)
kD	kilo Dalton(s)
kg	kilogram
L	litre(s)
LB	Luria Bertani
LIV	leucine, isoleucine and valine
LP	long pass
M.	*Mycobacterium*
m	milli- (10^{-3})
M	moles per litre

MA	mycolic acid
MCS	multi-cloning site
MDR	multi-drug resistant
min	minute(s)
MQW	Milli-Q purified water
Mtb	*Mycobacterium tuberculosis*
n	nano- (10^{-9})
NA	numerical aperture
N/A	not applicable
NaOAc	sodium acetate
NCBI	National Center for Biotechnology Information
NOC	nucleoid occlusion
OD	optical density
OEC	original environmental clone
ORF	open reading frame
P	probability
PBP	penicillin binding protein
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PG	peptidoglycan
pH	power of Hydrogen
psi	pounds per square inch

RNA	ribonucleic acid
ROS	reactive oxygen species
ROW	reverse osmosis purified water
rpm	revolutions per minute
s	second(s)
SD	standard deviation
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
sp.	species
SSC	side scatter – height (H), width (W)
TAE	tris acetate EDTA
TB	tuberculosis
TBE	tris borate EDTA
TDR	totally-drug resistant
TE	tris-EDTA buffer
tet	tetracycline
Tris	tris(hydroxymethyl)methylamine
U	units (of enzyme)
UV	ultraviolet
V	volt(s)
v/v	volume per volume
W	watt

WE	window extension
WHO	World Health Organisation
WT	wild-type
w/v	weight per volume
XDR	Extremely-drug resistant
µ	micro- (10^{-6})
µF	micro Farad

Abstract

Mycobacterium tuberculosis the causative organism of tuberculosis has been plaguing humanity for centuries. The number of effective antibiotics is dwindling due to the rise of multi-drug resistance within the species and new drugs need to be developed that target essential components of the bacterial life cycle. Bacterial cell division is an essential and highly conserved process across bacteria and new drugs that target this process could have broad-spectrum implications.

Bacilli can survive changes in their environment by forming filamentous cells, where cell division is inhibited while growth and DNA replication continue, giving rise to very long cells (up to 40 μm). Filamentation has been observed in both non-pathogenic and pathogenic bacteria, including *Escherichia coli* and *Mycobacterium tuberculosis* where it has been proposed to be required for replication and persistence within the human host. The process by which filamentation occurs in bacteria is not well understood. However, understanding filamentation can aid in identifying opportunities for new therapeutics and in addition, explore cell division in Mycobacteria as they are missing many of the key cell division genes present in model organisms like *Escherichia coli* and *Bacillus subtilis*.

The overall aim of this work was to use flow cytometry-based cell sorting to identify and characterize novel proteins that regulate cell division in Mycobacteria and allow persistence in mycobacterial disease. This was done by screening expression libraries of *Mycobacterium bovis* BCG genomic DNA (gDNA) hosted in *E. coli* and later *Mycobacterium smegmatis,* to identify clones expressing cell division proteins and regulatory genes via a filamentous phenotype.

The method for flow cytometry screening had to first be verified through the completion of a screen of a library of environmental DNA collected from the marine algae *Ulva australis*. Large environmental DNA inserts were sub-cloned and re-screened using flow cytometry-based cell sorting to identify genes causing filamentation when expressed. One reproducibly filamentous clone contained the Periplasmic Binding Protein Type-1 Superfamily conserved domain and we found that the overexpression of this gene caused a filamentous phenotype, which in turn showed that a single gene causing a filamentous phenotype could be identified with the flow cytometry based cell sorting method.

A library of *M. bovis* BCG gDNA was constructed and hosted in *E. coli*. This library was screened using flow cytometry-based cell sorting but no filamentous clones were found. The host species was then changed to *M. smegmatis* for better expression of heterologous genes and a modified expression vector utilizing the TET-ON/OFF inducible expression system was shown to work for the expression of cloned genes. Unfortunately after repeated attempts, a library of *M. bovis* BCG gDNA was unable to be constructed and screened for mycobacterial cell division genes and regulators.

Bacterial filamentation and cell division are important areas of investigation for clinically relevant bacteria. The information that can be gleaned from these investigations may lead to the next generation of antimicrobials.