Investigating Bacterial Filamentation as a Survival Strategy for Infection

by

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DOCTOR OF PHILOSOPHY

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Elizabeth Peterson, declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the 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.

This research is supported by the Australian Government Research Training Program.

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Peer-reviewed publications

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Publications in preparation

Helminth peptide therapy reduces *M. tuberculosis* induced inflammation. Giang H.B Le, Jessica Pedersen, Maxwell Stevens, Elizabeth Peterson, Nilesh J Bokil, Sheila Donnelly, Bernadette M Saunders

The role of bacterial size, shape and surface in macrophage engulfment of uropathogenic *E. coli* cells. Elizabeth Peterson, Bill Soderstrom, Nienke Prins, Giang H.B. Le, Lauren E. Hartley-Tassell, Chris Evenhuis, Rasmus Birkholm Grønnemose, Thomas Emil Andersen, Jakob Møller-Jensen, Gregory Iosifidis, Iain G. Duggin, Bernadette Saunders, Elizabeth J. Harry, Amy L. Bottomley.

Publications (not peer-reviewed)

Peterson, E. Bacterial filaments: not sick, but a survival strategy? *ASM NSW-ACT Branch Syntrophy* **22,** 4 (2021).

Conference and seminar proceedings

Shape-shifting bacteria are key to infection, e-Poster presentation at World Microbe Forum Conference, 2021.

Shape-shifting bacteria are key to infection, oral presentation at the Australian Society for Microbiology Annual Scientific Meeting, 2021.

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Prizes

ASM Nancy Millis Award Australian Society for Microbiology NSW-ACT 2021

Best Poster by a Student Lorne Infection and Immunity 2021

2nd Place Visualise Your Thesis University of Technology Sydney 2020

Winner Dr Loraine Holley Essay Prize University of Technology Sydney 2020

Runner-up Science Faculty 3 Minute Thesis University of Technology Sydney 2020

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°C	Degree Celsius
µF	Microfarad
µg/ml	Micrograms per millilitre
µl	Microlitres
µm	Micrometres
AMP	Ampicillin
ANOVA	Analysis of variance
cfu	Colony forming units
cfu/ml	Colony forming units per millilitre
CIP	Ciprofloxacin
CTX	Cefotaxime
DAPI	4′,6-diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal bovine serum
Fig	Figure
g	G-force
GFP	Green fluorescent protein
HMDM	Human monocyte derived macrophages
KAN	Kanamycin
kV	Kilovolts
LB	Lysogeny broth
LEX	Cephalexin
MEC	Mecillinam
mg/ml	Milligrams per millilitre
ml	Millilitre
mM	Micromolar
MOI	Multiplicity of infection
ms	Millisecond
msfGFP	Monomeric super folder green fluorescent protein
ng/ml	Nanograms per millilitre
OD	Optical density

p	*P*-value
PBS	Phosphate buffered saline
pH	Potential of hydrogen
rpm	Rotations per minute
RPMI	Roswell Park Memorial Institute
SEM	Standard error of the mean
SPT	Spectinomycin
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary tract infection
v/v	Volume per volume
w/v	Weight per volume
YFP	Yellow fluorescent protein
Ω	Ohm

Abstract

Numerous bacteria, both pathogenic and non-pathogenic, can change shape in response to environmental cues for survival; a phenomenon called morphological plasticity. Our understanding of this plasticity is based mainly on stress-free growth conditions in the laboratory, making it difficult to determine its role in bacterial responses to the pressures of 'real-world' environments. One such shape that has drawn research interest is that of filaments, formed predominately from rod-shaped bacteria such as *Escherichia coli.* Filamentous uropathogenic *E. coli* appear to play important roles in combating host defences during urinary tract infections through their decreased engulfment by phagocytic immune cells like macrophages. Bacterial filamentation, as a strategy to avoid engulfment during infections, is a possible virulence pathway that has been highlighted in previous research but is yet to be fully explored. Investigations in this area could reveal novel insights into bacterial-host interactions leading to the discovery of new targets for future treatments.

The aim of this thesis was to investigate how *E. coli* rods and filaments differ in their interactions with human macrophages. Conditions were established for creating viable populations of filamentous *E. coli* (strain UTI89) using antibiotics and genetic methods. Quantification of intracellular bacteria revealed that the longer the filament, the less likely it is to be engulfed. Changing the shape of UTI89 showed that spherical cells are more readily engulfed than rod-shaped cells of similar volume. However, engulfment of spheres decreases as volume increases, indicating that the shape of bacteria can influence engulfment to a certain extent before size and length become limiting.

The importance of bacterial surface was investigated by blocking macrophage mannose binding of UTI89 rods and filaments and using *fimH*-deleted UTI89. Surprisingly, while

blocking mannose binding resulted in reduced intracellular numbers of rods and filaments, deletion of *fimH* resulted in increased numbers and abolished macrophage length preference for rods. THP-1 macrophages and human monocyte derived macrophages (HMDMs) were used in this research. HMDMs behaved similarly to THP-1 macrophages, with the exception that HMDMs had detectable cytokine levels and their viability after infection with UTI89 rods and filaments differed. Significantly lower cytokine responses and viability were observed for HMDMs infected with filaments compared to those with rods.

This research has identified differences between UTI89 rods and filaments in the context of human macrophage interactions, providing a foundational understanding of filament engulfment. Eventually, this knowledge may reveal potential targets for the novel treatment of infections.