

# **Investigating Bacterial Filamentation as a Survival Strategy for Infection**

by

ELIZABETH MARGARET PETERSON

Thesis submitted in fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Under the supervision of Professor Liz Harry, Dr. Amy Bottomley and Associate

Professor Bernadette Saunders

University of Technology Sydney

Faculty of Science

August 2022

## **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.

**Signature:** Production Note:  
Signature removed prior to publication.

Date: 16/07/2022

---

## ACKNOWLEDGMENTS

I would like to firstly thank my supervisors Prof. Liz Harry, Dr. Amy Bottomley and Prof. Bernadette Saunders for their outstanding support during my PhD candidature. I am also extremely grateful to my i3 panel consisting of my supervisory team along with Iain Duggin and Diane McDougald. I would like to express my gratitude to past and present members of the Harry lab and Saunders lab for all the help and feedback they gave me. Thank you to Daniel, Matt, Nienke, Shirin, Riti, Connor, Izzy, Kat and Nural from the Harry lab. And thank you to Giang, Jess and Max from the Saunders lab. I would like to particularly thank Nienke Prins and Giang Le. Nienke assisted with completing the ciprofloxacin work for this project and Giang for teaching me tissue culture techniques, flow cytometry and isolation of monocytes from blood. I would also like to thank Bill Söderström for his collaboration by providing me with bacterial samples grown in his *in vitro* human bladder model.

I'd also like to thank past and present Microbial Imaging Facility (MIF) members Louise Cole, Chris Evenhuis and Amy, all of whom assisted greatly in teaching me microscopy techniques, providing expertise on various methods and experimental results, and Chris for building a script to analyse my images. Louise in particular has inspired a love of microscopy and using this technology as a quantification method not just for the pretty pictures we all know (and love). She kept me informed and encouraged me to attend many workshops regarding the new software and platforms available to researchers and I wouldn't have been able to expand my skillset as much as I did without her support. She also employed me for part of my PhD which was an amazing experience and enabled me to continue with my PhD in tough pandemic times when the usual casual work for PhD students wasn't available at the university.

Thank you to my wonderful family for supporting me through the ups and downs of this project and listening when my frustrations got the better of me. A shout out to my sister Georgia for reminding me of my end goal at least once a month by referring to me as ‘Dr. Liz’ (I can soon stop replying with “Not yet”). And of course, my parents for always supporting me no matter what, you two started me on this path long ago by encouraging my curiosity and love of learning. I’d like to also mention my Poppa. My Poppa was a talented man with a brilliant mind who enjoyed engaging his grandkids in scientific discussions no matter their age, which I think lead many of us to pursuing the sciences. He passed away during my Honours year and never got to see me make it this far, but I know he never doubted I would. Thank you.

To my friends who were always ready for a chat, to listen and sympathise, to share the woes of adulthood thank you so much. Kyna, Sarah and Tristan you’re the best bunch of nerds a girl could ask for. Evie thank you for moving back to Australia for me (you said it was the pandemic, but I know you really came for me). Evie also proofread my thesis out of the kindness of her heart.

And to all my other friends who always sounded super interested to hear what I have going on (even though my day-to-day life consisted of being in the lab with small breaks for sleeping and eating for three and a half years and was probably very boring to hear about) thank you and I hope to have new and exciting tales for you soon. Glenna and Giang, our shut up and write sessions may not have had a lot of ‘shutting-up’ but were very helpful in terms of writing, hopefully you guys can join me in finishing soon!

And to all the staff at AIMI, none of my research would be possible without the support you provide in the management and running of the facilities. Sarah Osvath deserves a mention by name for all the lab support she’s provided in terms of training, safety, and

immediate restocking of supplies that seemed to be mysteriously gone whenever I needed them most.

Finally, I'd like to thank the University of Technology Sydney and the Australian Government for supporting my research; this work was supported by the Australian Government Research Training Program (RTP) Stipend and RTP Fees Offset Scholarship.

The author acknowledges the use of the equipment: Zeiss Axioplan 2 microscope, DeltaVision Elite deconvolution microscope and OMX-SR microscope and associated software as well as Huygens image analysis software in the Microbial Imaging Facility at the Australian Institute for Microbiology and Infection (AIMI) in the Faculty of Science, the University of Technology Sydney. I would like to thank Louise Cole, Amy Bottomley and Chris Evenhuis for their scientific input and technical assistance.

## Peer-reviewed publications

Bottomley, A.L., Peterson, E., Iosifidis, G. *et al.* The novel *E. coli* cell division protein, YtfB, plays a role in eukaryotic cell adhesion. *Sci Rep* **10**, 6745 (2020). <https://doi.org/10.1038/s41598-020-63729-7>

## 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.

*Shape-shifting bacteria are key to infection*, e-Poster presentation at Lorne Infection and Immunity Conference, 2021.

*Shape-shifting bacteria are key to infection*, invited department presentation, Microbial Imaging Facility, The iThree Institute, University of Technology Sydney, 2020.

## 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

## TABLE OF CONTENTS

<b>Certificate of original authorship</b>	<b>ii</b>
<b>Acknowledgments</b>	<b>iii</b>
<b>Publications and awards</b>	<b>vi</b>
<b>Table of contents</b>	<b>viii</b>
<b>List of figures</b>	<b>xiv</b>
<b>List of tables</b>	<b>xix</b>
<b>List of abbreviations</b>	<b>xx</b>
<b>Abstract</b>	<b>xxii</b>
<b>Chapter 1 Introduction</b>	<b>1</b>
<b>1.1 Bacterial morphology</b>	<b>2</b>
1.1.1 Bacteria can change size and shape	2
1.1.2 The identification of bacterial filamentation which results from cell division inhibition	4
1.1.3 Antibiotic-induced filamentation	11
1.1.4 Bacterial filamentation during infections – the evolutionary path?	13
<b>1.2 Urinary tract infections</b>	<b>15</b>
1.2.1 A public health problem	15
1.2.2 The pathogenesis of urinary tract infections	16
<b>1.3 The immune response to invading bacterial pathogens</b>	<b>19</b>
1.3.1 Identifying pathogens and the innate immune response	19
1.3.2 Phagocytosis of bacteria by macrophages	22
1.3.3 Macrophage secretion of cytokines and chemokines	25
<b>1.4 Bacterial strategies to combat the immune response</b>	<b>26</b>
1.4.1 Avoiding detection	27
1.4.2 Manipulating the immune system	28
1.4.3 Filamentation to avoid engulfment...and more?	29
<b>1.5 Why do we need to understand the interactions of filamentous bacteria and our immune response?</b>	<b>31</b>
<b>1.6 Research aims and hypothesis</b>	<b>33</b>



<b>Chapter 2</b>	<b>Identifying conditions that produce viable, homogeneous populations of bacterial filaments</b>	<b>35</b>
<b>2.1</b>	<b>Introduction</b>	<b>36</b>
2.1.1	Filamentation is a deliberate change to cope with specific conditions	37
2.1.2	<i>Escherichia coli</i> and urinary tract infections as models for understanding filamentation	39
2.1.3	Chapter aims	41
<b>2.2</b>	<b>Materials and Methods</b>	<b>44</b>
2.2.1	Materials	44
2.2.2	Methods	48
<b>2.3</b>	<b>Results</b>	<b>54</b>
2.3.1	BW25113 cell length changes due to different environmental stimuli	54
2.3.2	UTI89 produces a homogeneous filamentous population with the addition of LEX	60
2.3.3	UTI89 produces a homogeneous filamentous population with the addition of CIP	66
2.3.4	Expression of <i>ftsZ-yfp</i> causes filamentation in UTI89	69
2.3.5	Populations of UTI89 filaments induced under different conditions are viable and reversible	73
<b>2.4</b>	<b>Discussion</b>	<b>89</b>
<b>Chapter 3</b>	<b>The role of UTI89 bacterial size, shape and surface in macrophage engulfment of uropathogenic <i>E. coli</i> cells</b>	<b>99</b>
	<b>Preface</b>	<b>100</b>
	<b>Abstract</b>	<b>102</b>
	<b>Introduction</b>	<b>103</b>
	<b>Results</b>	<b>106</b>
	Obtaining a population of viable UTI89 filaments of defined length using the cell division inhibitor, cephalixin	106
	Cephalixin- and ciprofloxacin-induced filamentation of <i>E. coli</i>	107
	UTI89 protects against engulfment by macrophages	

UTI89 filamentation induced by FtsZ expression also protects against engulfment by macrophages	110
Rods are preferentially engulfed by macrophages in mixed populations of rods and filaments	112
Is there a length threshold for rods above which macrophage engulfment decreases?	115
Macrophage engulfment is influenced by both shape and size of UTI89 cells	118
The reduced macrophage engulfment of filaments is unlikely to be due to changes in their ability to bind mannose	121
The preference for macrophages to engulf rods more readily than filaments is abolished by deleting <i>fimH</i>	124
Filaments from an <i>in vitro</i> human bladder infection model are also engulfed less effectively than rods by macrophages	127
UPEC filamentation occurs independently of the SOS-induced filamentation genes, <i>sulA</i> and <i>yfmM</i>	131
<b>Discussion</b>	<b>134</b>
<b>Methods</b>	<b>141</b>
Conditions to establish filamentation	141
Quantification of length and volume	143
Growth and culture of THP-1 monocytes	144
Gentamicin-protection assay	144
Imaging and deconvolution	145
Blocking with methyl $\alpha$ -D-mannopyranoside	146
Yeast agglutination assay	146
<i>In vitro</i> bladder infection model	147
Statistical analysis	148
<b>Supplementary Results</b>	<b>149</b>
Mannose binding as determined through yeast agglutination	159
Complementation of UTI89 $\Delta$ <i>fimH</i> did not restore wild-type engulfment	161
<b>Supplementary Methods</b>	<b>165</b>

	Strain construction – making mutants and electroporation of plasmids into strains	166
	Viability assays	167
	Glycan microarrays	169
<b>Chapter 4</b>	<b>THP-1 macrophage responses to infection with UTI89 rods and LEX-induced filaments</b>	<b>171</b>
<b>4.1</b>	<b>Introduction</b>	<b>172</b>
4.1.1	Phagocytosis dynamics of UPEC	173
4.1.2	Responses to infection with UPEC	175
4.1.3	Chapter aims	176
<b>4.2</b>	<b>Materials and Methods</b>	<b>177</b>
4.2.1	Materials	177
4.2.2	Methods	181
<b>4.3</b>	<b>Results</b>	<b>188</b>
4.3.1	Engulfment dynamics of rods and filaments using fluorescence microscopy	188
4.3.2	UTI89/pGI5 (msfGFP) rods and LEX-induced filaments are colocalised with different phagocytic proteins	206
4.3.3	The fate of internalised UTI89/pGI5 (msfGFP) rods and LEX-induced filaments	211
4.3.4	Interferon- $\gamma$ does not enhance the killing of intracellular UTI89/pGI5 (msfGFP) bacteria by macrophages	215
4.3.5	Cytokine and chemokine secretions of THP-1 macrophages infected with UTI89 (msfGFP) rods or LEX-induced filaments	217
4.3.6	Viability of THP-1 macrophages infected with UTI89 (msfGFP) rods or LEX-induced filaments	221
4.3.7	Actin-dependent blebbing of THP-1 macrophages in response to infection with UTI89/pGI5 (msfGFP) rods or LEX-induced filaments	227
<b>4.4</b>	<b>Discussion</b>	<b>235</b>

<b>Chapter 5</b>	<b>HMDM responses to infection with UTI89 (msfGFP) rods or LEX-induced filaments</b>	<b>243</b>
<b>5.1</b>	<b>Introduction</b>	<b>244</b>
5.1.1	The significance of replicating these experiments in HMDMs	245
5.1.2	Chapter aims	246
<b>5.2</b>	<b>Materials and Methods</b>	<b>236</b>
5.2.1	Materials	248
5.2.2	Methods	249
<b>5.3</b>	<b>Results</b>	<b>250</b>
5.3.1	HMDMs internalise higher numbers of UTI89/pGI5 (msfGFP) rods compared to LEX-induced filaments	250
5.3.2	HMDMs exhibit similar engulfment behaviour to THP-1 macrophages for UTI89/pGI5 (msfGFP) rods and LEX-induced filaments	251
5.3.3	The fate of internalised LEX-induced UTI89/pGI5 (msfGFP) filaments in HMDMs	257
5.3.4	UTI89/pGI5 (msfGFP) rods induce a stronger cytokine and chemokine response by HMDMs compared to LEX-induced filaments	259
5.3.5	HMDMs infected with LEX-induced UTI89/pGI5 (msfGFP) filaments have decreased metabolic viability	265
<b>5.4</b>	<b>Discussion</b>	<b>267</b>
<b>Chapter 6</b>	<b>General Discussion</b>	<b>271</b>
<b>6.1</b>	<b>The research goal</b>	<b>272</b>
<b>6.2</b>	<b>Filamentation as a survival advantage</b>	<b>273</b>
6.2.1	How does the size and shape of filaments influence macrophage engulfment?	273
6.2.2	Antibiotic-induced filaments versus filaments grown in a simulated bladder environment	275
<b>6.3</b>	<b>The importance of bacterial surface</b>	<b>276</b>
<b>6.4</b>	<b>The macrophage response to filaments</b>	<b>278</b>

6.4.1	The viability of filaments affects the metabolic activity of macrophages	278
6.4.2	Filament suppression of cytokines and chemokines	280
<b>6.5</b>	<b>The challenges of filamentation studies</b>	<b>283</b>
<b>6.6</b>	<b>The power of different models</b>	<b>291</b>
<b>6.7</b>	<b>Stress or evolution?</b>	<b>294</b>
<b>6.8</b>	<b>Concluding remarks</b>	<b>295</b>
	<b>Appendix</b>	<b>296</b>
	<b>Bibliography</b>	<b>337</b>

## List of figures

<b>Chapter 1</b>		
<b>Fig 1.1</b>	Formation of <i>E. coli</i> filaments.	<b>8</b>
<b>Fig 1.2</b>	SOS response in <i>E. coli</i> .	<b>10</b>
<b>Fig 1.3</b>	Urinary tract infection by uropathogenic <i>E. coli</i> (UPEC).	<b>18</b>
<b>Fig 1.4</b>	TLR4 recognition of LPS.	<b>21</b>
<b>Fig 1.5</b>	Macrophage phagocytosis of bacteria.	<b>24</b>
<b>Chapter 2</b>		
<b>Fig 2.1</b>	<i>E. coli</i> BW25113 cell length changes after growth at 37°C, 46°C and 8°C.	<b>56</b>
<b>Fig 2.2</b>	<i>E. coli</i> BW25113 filamentation in response to LEX and CTX.	<b>59</b>
<b>Fig 2.3</b>	Increasing the concentration of LEX increases the cell length of UTI89.	<b>63</b>
<b>Fig 2.4</b>	Three populations of UTI89 chosen for further study: untreated rods, rods treated with 2.5 µg/ml LEX and filaments induced by 10 µg/ml LEX.	<b>65</b>
<b>Fig 2.5</b>	Increasing the concentration of CIP increases the cell length of UTI89.	<b>68</b>
<b>Fig 2.6</b>	Cell length increases when <i>ftsZ-yfp</i> is expressed from a plasmid.	<b>72</b>
<b>Fig 2.7</b>	Reversion of UTI89 filamentous populations occurs on removal of LEX.	<b>75</b>
<b>Fig 2.8</b>	Reversion of UTI89 filamentous populations occurs on removal of CIP.	<b>77</b>
<b>Fig 2.9</b>	Reversion of UTI89/pLau80 filamentous populations occurs on removal of arabinose.	<b>79</b>
<b>Fig 2.10</b>	Correlation between cfu and biomass for UTI89 Rods and LEX filaments.	<b>82</b>
<b>Fig 2.11</b>	Membrane permeability of rods and filaments induced by LEX, CIP and <i>ftsZ-yfp</i> expression.	<b>84</b>
<b>Fig 2.12</b>	Metabolic viability of rods and filaments induced by LEX, CIP and <i>ftsZ-yfp</i> expression.	<b>87</b>

### Chapter 3

<b>Fig 1</b>	THP-1 macrophages engulf UTI89 filaments significantly less than rods.	<b>109</b>
<b>Fig 2</b>	In a mixed populations of rods and filaments, rods are engulfed by THP-1 macrophages preferentially over filaments.	<b>114</b>
<b>Fig 3</b>	THP-1 macrophage engulfment of UTI89 decreases exponentially as bacterial size increases.	<b>117</b>
<b>Fig 4</b>	THP-1 macrophages have bacterial size and shape preferences.	<b>120</b>
<b>Fig 5</b>	Blocking mannose binding with methyl $\alpha$ -D-mannopyranoside reduces THP-1 macrophage engulfment of UTI89 rods and filaments	<b>123</b>
<b>Fig 6</b>	Deleting <i>fimH</i> can override the THP-1 macrophage preference to engulf rods more effectively than filaments.	<b>126</b>
<b>Fig 7</b>	The preference for THP-1 macrophage engulfment of rods over filaments is maintained for UTI89/pGI5 (msfGFP) isolated from an <i>in vitro</i> human bladder model.	<b>129</b>
<b>Fig 8</b>	The preference for THP-1 macrophage engulfment of rods over filaments is maintained for UTI89 $\Delta$ <i>sulA</i> $\Delta$ <i>yfmM</i> /pGI5 (msfGFP) isolated from an <i>in vitro</i> human bladder model.	<b>133</b>
<b>Fig S1</b>	Phase contrast images of rods and filaments induced by cephalixin, ciprofloxacin and <i>ftsZ-yfp</i> expression.	<b>149</b>
<b>Fig S2</b>	Membrane permeability of rods and filaments induced by cephalixin, ciprofloxacin and <i>ftsZ-yfp</i> expression.	<b>151</b>
<b>Fig S3</b>	Metabolic viability of rods and filaments induced by cephalixin, ciprofloxacin and <i>ftsZ-yfp</i> expression.	<b>152</b>
<b>Fig S4</b>	Reversion of UTI89 filamentous populations occurs on removal of cephalixin, ciprofloxacin or FtsZ-YFP inducer.	<b>153</b>
<b>Fig S5</b>	UTI89/pGI5 (msfGFP) LEX filaments do not revert significantly to rods during a 1-hour incubation in RPMI culture media.	<b>154</b>

<b>Fig S6</b>	Correlation between cfu and biomass for UTI89 Rods and LEX Filaments.	<b>155</b>
<b>Fig S7</b>	The addition of a centrifuging, wash and dilution step in growth methods of UTI89/pLau80 is responsible for reduced macrophage engulfment of FtsZ Rods.	<b>157</b>
<b>Fig S8</b>	Engulfment of complemented UTI89 $\Delta$ <i>fimH</i> does not restore wild-type levels of macrophage engulfment.	<b>162</b>
<b>Fig S9</b>	Macrophage engulfment of UTI89 $\Delta$ <i>fimH</i> does not depend on mannose binding.	<b>163</b>
<b>Fig S10</b>	Lengths of bacteria isolated from the <i>in vitro</i> human bladder model.	<b>164</b>
<b>Chapter 4</b>		
<b>Fig 4.1</b>	Digital analysis of microscopy images of engulfment with FIJI software.	<b>186</b>
<b>Fig 4.2</b>	Rods are engulfed more effectively by macrophages than filaments.	<b>190</b>
<b>Fig 4.3</b>	Representative 3-D image of THP-1 macrophages with UTI89/pGI5 (msfGFP) rods 30-minutes post-infection.	<b>193</b>
<b>Fig 4.4</b>	Representative 3-D image of THP-1 macrophages with UTI89/pGI5 (msfGFP) filaments 30-minutes post-infection.	<b>194</b>
<b>Fig 4.5</b>	UTI89/pGI5 (msfGFP) LEX-induced filaments are engulfed less effectively by macrophages compared to rods.	<b>195</b>
<b>Fig 4.6</b>	UTI89/pGI5 (msfGFP) dead filaments are engulfed more by macrophages at 60 minutes compared to live filaments but are internalised at the same percentage.	<b>198</b>
<b>Fig 4.7</b>	UTI89 $\Delta$ <i>fimH</i> /pGI5 (msfGFP) rods and UTI89 $\Delta$ <i>fimH</i> /pGI5 (msfGFP) filaments are engulfed similarly by macrophages.	<b>201</b>
<b>Fig 4.8</b>	UTI89/pGI5 (msfGFP) bladder rods from the <i>in vitro</i> bladder model are fully internalised more efficiently by macrophages over filaments, but more macrophages attempt to engulf filaments.	<b>204</b>
<b>Fig 4.9</b>	UTI89/pGI5 (msfGFP) rods and LEX-induced filaments grown in LB and combined after growth show no	<b>205</b>



	differences between the percentage of macrophages infected and the number of bacteria per macrophage.	
<b>Fig 4.10</b>	Phagocytic proteins localise with UTI89/pGI5 (msfGFP) rods at a higher frequency than LEX-induced filaments in THP-1 macrophages.	<b>209</b>
<b>Fig 4.11</b>	LEX-induced filaments can redivide into viable rods inside THP-1 macrophages.	<b>213</b>
<b>Fig 4.12</b>	Intracellular LEX-induced filaments revert to rods inside macrophages.	<b>214</b>
<b>Fig 4.13</b>	IFN $\gamma$ does not enhance the killing of intracellular UTI89/pGI5 (msfGFP) by THP-1 macrophages.	<b>216</b>
<b>Fig 4.14</b>	Cytokine response of macrophages to UTI89/pGI5 (msfGFP) rods and LEX-induced filaments.	<b>219</b>
<b>Fig 4.15</b>	Chemokine response of macrophages to UTI89/pGI5 (msfGFP) rods and LEX-induced filaments.	<b>220</b>
<b>Fig 4.16</b>	THP-1 macrophage populations infected with LEX-induced filaments have decreased membrane integrity	<b>223</b>
<b>Fig 4.17</b>	The metabolic viability of THP-1 macrophage populations after infection with UTI89/pGI5 (msfGFP) live bacteria, dead bacteria or UTI89 $\Delta$ <i>fimH</i> /pGI5 (msfGFP).	<b>226</b>
<b>Fig 4.18</b>	Time-lapse microscopy of infected THP-1 macrophages shows blebbing phenomenon.	<b>230</b>
<b>Fig 4.19</b>	Blebs of THP-1 macrophages infected with UTI89/pGI5 (msfGFP) rods contain F-actin.	<b>231</b>
<b>Fig 4.20</b>	Blebs of THP-1 macrophages infected with LEX-induced filaments contain F-actin.	<b>232</b>
<b>Fig 4.21</b>	No blebs containing F-actin were observed with uninfected THP-1 macrophages, and less concentrated actin was observed at macrophage edges.	<b>233</b>
<b>Chapter 5</b>		
<b>Fig 5.1</b>	HMDMs internalise more UTI89/pGI5 (msfGFP) rods than LEX-induced filaments.	<b>251</b>

<b>Fig 5.2</b>	Representative 3-D images of HMDMs with UTI89/pGI5 (msfGFP) rods 30-minutes post infection.	<b>253</b>
<b>Fig 5.3</b>	Representative 3-D images of HMDMs with LEX-induced UTI89/pGI5 (msfGFP) filaments 30-minutes post infection.	<b>254</b>
<b>Fig 5.4</b>	LEX-induced UTI89/pGI5 (msfGFP) filaments are less effectively internalised by HMDMs compared to rods, and there are more HMDMs engulfing rods than filaments.	<b>255</b>
<b>Fig 5.5</b>	Comparison of engulfment behaviour of THP-1 macrophages and HMDM incubated with UTI89 rods or filaments for 60 minutes.	<b>256</b>
<b>Fig 5.6</b>	HMDMs reduce engulfed levels of UTI89/pGI5 (msfGFP) rods and LEX-induced filaments to 1% after 48 hours.	<b>258</b>
<b>Fig 5.7</b>	The cytokine response of HMDMs to UTI89/pGI5 (msfGFP) rods is above basal levels for TNF- $\alpha$ , IL-6 and CCL3.	<b>262</b>
<b>Fig 5.8</b>	HMDMs produce more TNF- $\alpha$ and IL-6 when infected with UTI89/pGI5 (msfGFP) rods compared to LEX-induced filaments.	<b>263</b>
<b>Fig 5.9</b>	HMDMs infected with LEX-induced UTI89/pGI5 (msfGFP) filaments produce the same amount or less of TNF- $\alpha$ , IL-6, CCL2 and CCL3 compared to uninfected HMDMs.	<b>264</b>
<b>Fig 5.10</b>	HMDMs infected with LEX-induced UTI89/pGI5 (msfGFP) filaments have reduced metabolic viability compared to those infected with UTI89/pGI5 (msfGFP) rods.	<b>266</b>
<b>Appendix</b>		
<b>Supplementary information 2</b>	Comparison of THP-1 engulfment of formaldehyde killed UTI89/pGI5 (msfGFP) rods and LEX-induced UTI89/pGI5 (msfGFP) filaments.	<b>335</b>
<b>Supplementary information 3</b>	Comparison of THP-1 engulfment of UTI89/pGI5 (msfGFP) filaments from the <i>in vitro</i> human bladder model and LEX-induced UTI89/pGI5 (msfGFP) filaments.	<b>336</b>

## List of tables

<b>Chapter 1</b>		
<b>Table 1.1</b>	Range of bacterial species that undergo filamentation in response to different environments.	<b>6</b>
<b>Chapter 2</b>		
<b>Table 2.1</b>	Solutions and media.	<b>44</b>
<b>Table 2.2</b>	Antibiotics and chemicals.	<b>44</b>
<b>Table 2.3</b>	Plasmids used in this chapter	<b>45</b>
<b>Table 2.4</b>	Strains used or constructed in this chapter.	<b>46</b>
<b>Table 2.5</b>	Commercial kits.	<b>47</b>
<b>Table 2.6</b>	Average cell lengths of UTI89 exposed to increasing concentrations of LEX.	<b>62</b>
<b>Chapter 3</b>		
<b>Table S1</b>	Glycan binding profile of UTI89 Rods, LEX Rods and LEX Filaments.	<b>158</b>
<b>Table S2</b>	Agglutination of yeast cells with bacterial cultures of 2-fold dilutions.	<b>160</b>
<b>Table S3</b>	<i>E. coli</i> strains and plasmids used in this study.	<b>165</b>
<b>Table S4</b>	Primers used in this study.	<b>166</b>
<b>Chapter 4</b>		
<b>Table 4.1</b>	Solutions and media.	<b>177</b>
<b>Table 4.2</b>	Dyes and antibodies.	<b>177</b>
<b>Table 4.3</b>	Antibiotics and chemicals.	<b>178</b>
<b>Table 4.4</b>	Commercial kits.	<b>178</b>
<b>Table 4.5</b>	Plasmids used in this chapter.	<b>180</b>
<b>Table 4.6</b>	Strains used or constructed in this chapter	<b>180</b>
<b>Chapter 5</b>		
<b>Table 5.1</b>	Solutions and media.	<b>248</b>
<b>Table 5.2</b>	Antibiotics and chemicals.	<b>249</b>
<b>Table 6.1</b>	Advantages and disadvantages of methods utilised in filamentation studies.	<b>284</b>

## List of abbreviations

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

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