Defining the *Clostridium difficile spo0A* regulon and its role in disease and transmission

By

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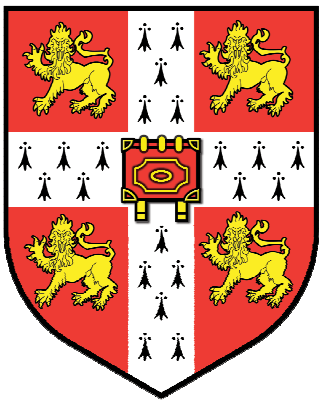
2012



This dissertation is submitted for the degree of
Doctor of Philosophy

Darwin College
University of Cambridge

Abstract

Clostridium difficile is an intestinal dwelling bacterium that is a major cause of antibiotic-associated diarrhoea, representing a major healthcare-associated problem and significant economic burden. Unlike most other healthcare pathogens, *C. difficile* produces highly infective and resistant spores that are excreted by infected patients, producing an environmental transmission reservoir that confounds standard disinfection regimens. Thus, infection with *C. difficile* is now endemic in many hospitals.

The aim of this study was to assess the role of the *C. difficile spo0A* gene in disease and transmission, and to identify the genes under the control of Spo0A, that is, its regulon. Here, we use a murine model of infection to examine the role of the *C. difficile spo0A* gene during infection and transmission. We demonstrate that *C. difficile spo0A* mutant derivatives cause exaggerated virulence in mice, linked to an increase in the production of toxins A and B, but that these mutant derivatives are unable to persist within and effectively transmit between mice. Thus, the *C. difficile* Spo0A protein plays a key role in persistent infection, including recurrence and host-to-host transmission in this model. This data has potential clinical implications related to the management of hospital patients.

The *C. difficile spo0A* gene encodes for a highly conserved transcriptional regulator of sporulation. Here, we define the *C. difficile spo0A* regulon using transcriptomic and proteomic analyses. We validate Spo0A as a regulator of a number of sporulation genes and confirm that Spo0A negatively regulates toxin production. Spo0A also negatively regulates key components of the *C. difficile* flagellar assembly apparatus and modulates several metabolic pathways, including the fermentation of carbohydrates leading to the production of butyrate.

Thus, the *C. difficile spo0A* gene is a global transcriptional regulator that coordinates multiple virulence, sporulation and metabolic phenotypes during *C. difficile* disease and transmission.

Acknowledgements

First and foremost I would like to thank my supervisor Prof. Gordon Dougan for giving me the opportunity to complete this Ph.D in his laboratory, as well as for his ongoing support and expert supervision. I also extend my gratitude to my mentor, Dr Trevor Lawley for his direction and guidance throughout this work. Additionally, I wish to acknowledge the Wellcome Trust, whose generous funding has underwritten this work.

I would also like to make a special mention of those who helped me during parts of my Ph.D, and without whom this work could not have been completed: Dr Simon Clare for his expert ideas, insights and knowledge of mouse infections, David Goulding for his incredible EM images, Dr Derek Pickard for his interesting approach to molecular microbiology, and Lars Barquist and Melissa Martin for teaching me the ways of ssRNA-Seq.

To all the other people who encouraged, supported and advised me during my Ph.D, I cannot list you all, but thank you for everything.

A massive thanks to everyone in Team 15 is also deserved, for their willingness to give their time, expertise and support. I look forward to seeing you all again!

Finally, I would like to thank my family for their unconditional and ongoing support. I dedicate this thesis to my parents, Andrew and Susan, for never doubting that I could do it, and to my wonderful husband Charles, for his endless reassurance, support and encouragement. I truly could not have done it without you.

Declaration

I hereby declare that this thesis is the result of my own work and includes no material written by any other person or material which is the outcome of work done in collaboration except where specifically indicated in here or in the Materials and methods section. I was fully involved in all aspects of the design and experimental work associated with this thesis.

Dr Simon Clare (Wellcome Trust Sanger Institute, Cambridge), assisted the author in performing tissue collection from experimental animals and performed mouse immunisations. Mr David Goulding (Wellcome Trust Sanger Institute, Cambridge) performed Transmission Electron Microscopy, in assistance with the author. Mass spectrometry was performed by the core proteomics facility (Wellcome Trust Sanger Institute, Cambridge) in assistance with the author. The analysis of the results was performed by the author. Transcript mapping was performed by the core pathogen informatics team as part of the ssRNA-Seq mapping pipeline (Wellcome Trust Sanger Institute, Cambridge). The analysis of the results was performed by the author.

C. difficile 630 Δ*spo0A* and R20291Δ*spo0A* mutants were generated by Dr Lisa Dawson (London School of Hygiene and Tropical Medicine, London) and Dr Robert Fagan (Imperial College, London), respectively. Butyrate quantification was performed in collaboration with Dr Sylvia Duncan (Rowett Institute of Nutrition and Health, Aberdeen). Spo0A purification was performed by Dr Wiep Klass Smits (Leiden University, Leiden).

None of the material presented herein has been submitted previously for the purpose of obtaining another degree. This thesis does not exceed 60,000 words, as required by the School of Biological Sciences.

Laura J. Pettit
October, 2012

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List of abbreviations

BYA	Billion years ago
CDAI	*Clostridium difficile*-associated infection
cDNA	Complementary DNA
CDS	Coding sequence
CI	Competitive index
CFU	Colony forming units
Cy3	Cyanine-3
DAPI	4', 6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
DOH	Department of Health
DPA	Dipicolinic acid
ELISA	Enzyme-linked immunosorbent assay
FDR	False discovery rate
FITC	Fluorescein isothiocyanate
FRT	Faecal replacement therapy
HPA	Health Protection Agency
IAA	Idoacetamide
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IGEM	ImmunoGold electron microscopy
IL	Interleukin
IBD	Inflammatory bowel disease
IPTG	Isopropyl-β-d-thiogalactopyranoside
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LCT	Large clostridial toxin
LTCF	Long-term care facility
NAP1	North American Pulsotype 1
NHS	National Health Service

ONS	Office for National Statistics
PaLoc	Pathogenicity locus
PCR	Polymerase chain reaction
PMC	Pseudomembranous colitis
PMN	Polymorphonuclear neutrophil
PPI	Proton pump inhibitor
PTS	Phosphotransferase system
RAM	Retrotransposition-activated marker
REA	Restriction endonuclease analysis
RNA	Ribonucleic acid
SASP	Small acid-soluble protein
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SCFA	Short-chain fatty acid
SOE PCR	Splicing by overlapping extension polymerase chain reaction
ssRNA-Seq	Strand-specific cDNA sequencing
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
TCEP	Tris(2-carboxyethyl)phosphine
TEAB	Triethylammonium bicarbonate
TEM	Tranmission electron microscopy
TLR	Toll-like receptor
TMB	3, 3', 5, 5'-tetramethylbenzidine
TNF-α	Tumour necrosis factor-alpha
WTSI	Wellcome Trust Sanger Institute