Ph.D. Thesis

Understanding bacterial adaptation to aerobic and anaerobic environments through experimental evolution and whole genome analysis

Thomas Finn

December 2014

A thesis presented in fulfilment of the requirements for the degree of Doctor of Philosophy in Genetics at Massey University, Palmerston North, New Zealand

Abstract

Facultative anaerobic organisms have the metabolic versatility to grow in both aerobic and anaerobic environments. However, molecular mechanisms that underpin adaptation to anaerobic environments are not well understood. This study aims to understand how the facultative anaerobe, *Escherichia coli*, adapts to environments that vary in oxygen content. An experimental evolution experiment was conducted in which replicate lineages were established from a pre-evolved clonal culture of *E. coli* REL4536. Lineages were serially sub-cultured for 4,000 generations within strict aerobic and strict anaerobic environments, and a treatment that fluctuated between the two environments. Significant increases in the relative fitness of lineages exposed to anaerobic conditions were observed, whereas the relative fitness of lineages in aerobic conditions did not increase, likely as the ancestor had been pre-adapted to aerobic growth.

Mutations that arose during evolution were identified by genome sequencing randomly-selected clones from each lineage at 2,000 and 4,000 generations. Traits that contributed to adaptation were predicted via the occurrence of independent mutations affecting common traits among lineages. Adaptation to the anaerobic environment was facilitated by modifications to anaerobic fermentation and the inactivation of virulence genes, whereas in the aerobic environment, mutations predicted to confer a growth advantage in stationary phase were observed. The evolution of generalists involved traits that were similar to those found in both aerobic and anaerobically evolved lineages, as well as the deletion of cryptic prophages from the genome and modifications to amino acid transport.

Phenotypically distinct small colony morphotypes (SCM) arose within anaerobic lineages and two separate adaptive pathways are hypothesised for this divergence. SCM1 were capable of stable co-existence with co-evolved cells of typical colony morphotype, most likely through an acetate cross-feeding mechanism. In contrast, SCM2 was able to out-compete the ancestor within 14 days, despite exhibiting a lower growth rate than the ancestor. SCM2 likely evolved the ability to inhibit the ancestral strain through a contact dependent inhibition mechanism, as evidenced by a mutation in *glgC*. This thesis demonstrates the complex nature of adaptation to anaerobic environments, as revealed by experimental evolution and whole genome sequencing.

Acknowledgements

First and foremost, I would like to thank my supervisors, Dr Christina Moon, Dr Sinead Leahy, Dr Rees Kassen and Prof Paul Rainey. Your advice and insights into this project were invaluable. In particular Christina and Sinead, your guidance, support and constant encouragement over the years have been appreciated every second of the way. Giving me the opportunity to partake in this project brought me to New Zealand, and represents the beginning of my own adult life, and for that I am forever grateful to you both.

I would like to acknowledge AgResearch Ltd and Massey University for providing financial assistance and an excellent working environment during the course of my studies. I would also like to thank the Marsden Fund for providing funding for this project.

I would like to thank a great bunch of people, collectively known as the Rumen Microbiology team at AgResearch, Grasslands. Working amongst you all has been a great pleasure. In particular thanks to Dr Bill Kelly, Dr Suzanne Lambie, Dr Gemma Henderson for assistance during my first year. Thanks also to Dr Peter Janssen and Dr Ron Ronimus for helpful discussions, and Dr Dragana Gagic for her sense of humour. For technical assistance in the lab, thank you Carrie Sang, Dong Li and Dr Sam Noel and especially Rechelle Perry, for her friendship and showing me the ropes with what would become my beloved anaerobic chamber. Outside of the Rumen Microbiology team, thank you also to Dr Mark McCann, Paul Mclean, Paul Lovejoy and Denise Martin. To all those in the AgResearch student room, both past and present, the daily laughs and therapy sessions we enjoyed while sitting around the student table in the AgReseach café kept me hanging in there.

To Dr Milica Ciric, your friendship has meant the world to me. To Sonal Shewaramani and Renee Atua, I cannot think of anyone else who would have made the last few years as much fun as they were, thank you both for that, and here's to many more! To Jamie Lange, thank you for consistently being so dependable, understanding and supportive throughout this process. Without encouragement from you four, I would never have reached this point.

Lastly, I would like to dedicate this thesis to my parents Tom and Collette Finn, and brother, Jim. Being so far away from you has been hard on us all. I hope you can see it as a necessary step towards the only goal that matters to me; to make you guys proud.

Table of contents

List of figures

List of tables

Non-standard abbreviations

Abbreviation	Meaning
Acetyl-CoA	Acetyl coenzyme A
AE	Aerobic
AN	Anaerobic
Anc	Ancestor
AP	Antagonistic pleiotropy
ATP	Adenosine triphosphate
bp	Base pairs
ca	Circa
cAMP	Cyclic adenosine monophosphate
CFU	Colony forming units
d	Day
DNA	Deoxyribonucleic acid
FL	Fluctuating
FSW	Fast switcher
FS	Fuzzy spreader
g	Gravity
GASP	Growth advantage in stationary phase
Gb	Gigabase pairs
GCR	Gross chromosomal rearrangements
hrs	Hours
IS	Insertion element
K	Thousand
kb	Kilo bases
L	Litre
LG	Large
LTEE	Long-term experimental evolution
M	Molar
MA	Mutation accumulation
Mb	Megabase pairs
MP	Mate pair
NADH	Nicotinamide adenine dinucleotide
NFDS	Negative frequency dependent selection
NGS	Next generation sequencing
nm	Nanometers
OD	Optical density
PCR	Polymerase chain reaction
PE	Paired end
PEP	Phosphoenolypyruvate
ppGpp	Guanosine pentaphosphate
Rif^r	Rifampicin resitent reference strain
RNA	Ribonucleoic acid
ROS	Reactive oxygen species
rpm	Rotations per minute
SCDI	Stationary phase contact-dependent inhibition
SCM	Small colony morphotype

SCV	Single colony variant
SL	Small
SM	Smooth
SNP	Single nucleotide polymorphisms
SSW	Slow switcher
TA	Toxin-antitoxin
TAra	Tetrazolium and arabinose
TCA	Tricarboxylic acid
TCM	Typical colony morphotype
U	Units
UPEC	Urethropathogenic
UV	Ultra violet
WGS	Whole genome sequencing
WS	Wrinkly spreader
wt/vol	Weight/volume