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Ph.D. Thesis

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

Thomas Finn

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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.

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Table of contents

Abstract	iii
Acknowledgements	v
Table of contents	vii
List of figures	xv
List of tables	xvii
Non-standard abbreviations	xix
Chapter One : Literature review	1
1.1. Introduction	1
1.1.1. Growth in an aerobic environment.....	2
1.1.2. Anaerobic growth	3
1.1.3. Fluctuating between aerobic and anaerobic growth in <i>E. coli</i>	5
1.1.4. Impact of aerobic and anaerobic metabolism on the genome	7
1.2. Overview of evolutionary processes.....	8
1.2.1. Origins of genetic variation.....	8
1.2.1.1. Types of mutations	9
1.2.1.1.1. Prevalence of mutations in aerobic and anaerobic environments.....	10
1.2.1.1.2. Ecological effects of mutations.....	11
1.2.1.1.2.1. Classification of beneficial mutations	11
1.2.2. Genetic drift	12
1.2.3. Genetic draft	12
1.2.4. Natural selection	12
1.2.4.1. Frequency dependent selection	13
1.3. Outcomes of evolution	13
1.3.1. Adaptation	14
1.3.1.1. The adaptive landscape	14
1.3.2. The evolution of specialists.....	15
1.3.3. The evolution of generalists.....	17
1.3.4. The evolution of biodiversity	17
1.4. Empirical studies on adaptive evolution	18
1.4.1. Rate of adaptive evolution from LTEE studies	19
1.4.2. Identification of adaptive mutations from LTEE studies.....	19

1.4.2.1. Examples of adaptive mutations from LTEE studies	20
1.4.2.1.1. Enhanced DNA supercoiling	21
1.4.2.1.2. Modification of the stringent response.....	22
1.4.2.1.3. Decreased cell wall biosynthesis	22
1.4.2.1.4. Glucose specificity	23
1.4.2.1.5. Resource switching.....	23
1.4.2.1.5.1. Growth advantage in stationary phase	25
1.4.2.1.6. Findings from LTEE studies varying oxygen exposure	25
1.5. Sympatric diversification	26
1.5.1. Diversification in response to spatial heterogeneity.....	27
1.5.2. Diversification within chemostat cultures on single substrate media	28
1.5.3. Diversification within batch cultures with mixed acetate and glucose media.....	29
1.5.4. Diversification within the glucose utilising population of <i>E. coli</i>	30
1.6. Combining LTEE with whole genome sequencing to explore the adaptive landscape	30
1.7. Thesis Outline	33
Chapter Two : Materials and methods	37
2.1. Materials.....	37
2.1.1. Lab equipment.....	37
2.1.2. Lab chemicals and enzymes	38
2.1.2.1. DNA-free water.....	38
2.1.2.2. PCR reagents.....	38
2.1.2.3. Ribonuclease A	38
2.1.2.4. Proteinase K.....	38
2.1.3. Bacterial strains	39
2.1.4. Solutions and buffers.....	39
2.1.4.1. Tris-HCl	39
2.1.4.2. TE buffer	39
2.1.4.3. 50× TAE buffer	39
2.1.4.4. 5 M NaCl solution	39
2.1.4.5. 5% (wt/vol) Triphenyltetrazolium chloride solution	39
2.1.4.6. Crystal violet staining reagent	40
2.1.4.7. Gram's Iodine	40

2.1.4.8. Decolorising agent	40
2.1.4.9. Safranin	40
2.1.4.10. Ethanol	40
2.1.4.11. Isopropanol	40
2.1.4.12. Liquid N ₂	40
2.1.4.13. Lysis buffer	40
2.1.4.14. EDTA.....	40
2.1.4.15. SDS solution	41
2.1.4.16. Phenol:chloroform: isoamyl alcohol solution	41
2.1.4.17. Chloroform isoamyl alcohol solution	41
2.1.4.18. Sodium acetate solution	41
2.1.4.19. 1× phosphate buffered saline	41
2.1.4.20. L-cysteine-HCl reducing agent	41
2.1.5. Liquid media.....	41
2.1.5.1. Lysogenic-Broth (LB) medium	41
2.1.5.1.1. Aerobic preparation.....	41
2.1.5.1.2. Anaerobic preparation	42
2.1.5.2. Davis minimal (DM) medium	42
2.1.5.2.1. Aerobic salt preparation	42
2.1.5.2.1.1. Aerobic additive addition.....	42
2.1.5.2.2. Anaerobic preparation.....	42
2.1.5.2.2.1. Anaerobic additive addition.....	43
2.1.6. Solid media.....	43
2.1.6.1. LB solid medium.....	43
2.1.6.1.1. Aerobic preparation.....	43
2.1.6.1.2. Anaerobic preparation	43
2.1.6.2. DM solid medium.....	43
2.1.6.2.1. Aerobic preparation.....	43
2.1.6.2.2. Anaerobic preparation.....	44
2.1.6.3. Minimal arabinose (MA) solid medium	44
2.1.6.4. Tetrazolium arabinose (TA) solid medium	44
2.1.7. Media additives.....	45

2.1.7.1. DM25 additives.....	45
2.1.7.1.1. Aerobic preparation	45
2.1.7.1.1.1. Glucose	45
2.1.7.1.1.2. Magnesium sulphate	45
2.1.7.1.1.3. Thiamine	45
2.1.7.1.2. Anaerobic preparation	45
2.1.7.2. 70% glycerol saline	45
2.1.7.2.1. Aerobic preparation	45
2.1.7.2.2. Anaerobic preparation	45
2.1.7.3. Antibiotics.....	46
2.2. Methods	46
2.2.1. Growth conditions	46
2.2.1.1. Aerobic cultures.....	46
2.2.1.2. Anaerobic cultures.....	46
2.2.2. Culture resuscitation	47
2.2.3. Growth courses.....	47
2.2.4. Gram stains.....	48
2.2.5. Long-term adaptation experiment	48
2.2.5.1. Establishment of long term evolving populations.....	48
2.2.5.2. Maintenance of evolving populations.....	49
2.2.5.3. Cell density monitoring of evolving populations.....	50
2.2.5.4. Contamination checks of evolving populations	50
2.2.5.5. Storage of evolving populations.....	51
2.2.6. Competitive fitness assays.....	51
2.2.6.1. Generation of spontaneous Ara ⁺ mutant strains.....	51
2.2.6.2. Generation of antibiotic resistant mutant strains.....	51
2.2.7. Fitness assays with Ara ⁺ marker	52
2.2.8. Fitness assays with Rif ^r marker	52
2.2.8.1. Fitness calculation	53
2.2.9. Reciprocal invasion assay	54
2.2.10. Cross-feeding assay	55
2.2.10.1. Media preparation.....	55

2.2.10.2. Assay	55
2.2.11. Polymerase chain reaction.....	55
2.2.11.1. Primers	55
2.2.11.2. Reactions.....	56
2.2.11.3. Agarose gel electrophoresis.....	56
2.2.11.4. PCR purification.....	57
2.2.11.5. Colony PCR	57
2.2.12. DNA extractions	57
2.2.12.1. DNA extractions using phenol:chloroform	57
2.2.13. DNA quantification.....	58
2.2.14. Whole genome sequencing	58
2.2.15. Illumina sequencing	59
2.2.16. Mutation identification.....	59
2.2.17. Bioinformatic resources and software.....	60
2.2.18. Statistical analysis	61
Chapter Three : Adaptation to aerobic and anaerobic environments	63
3.1. Introduction	63
3.2. Objectives.....	64
3.3. Results and discussion	64
3.3.1. Growth dynamics of REL4536 in batch cultures in aerobic and anaerobic environments	64
3.3.1.1. Citrate utilisation of REL4536 in the anaerobic environment	66
3.3.2. Establishment of long-term lineages in aerobic, anaerobic and fluctuating treatments	69
3.3.3. Assessment of fitness of evolving populations.....	71
3.3.3.1. Development of neutrally marked strains for competitive fitness assays	71
3.3.3.1.1. Neutrality of Ara+ marked strains under aerobic and anaerobic conditions ...	71
3.3.3.1.2. Neutrality of antibiotic resistance markers under aerobic and anaerobic conditions.....	73
3.3.3.2. Adaptation to narrow niches	75
3.3.3.2.1. Evolution of the aerobic lineages – general trends	75
3.3.3.2.1.1. Evolution of the aerobic lineages – individual lineage trends	77
3.3.3.2.2. Evolution of the anaerobic lineages – general trends	79

3.3.3.2.2.1. Evolution of the anaerobic lineages – individual lineage trends.....	81
3.3.3.3. Adaptation to a broad niche.....	83
3.3.3.3.1. Evolution of the fluctuating lineages in aerobic and anaerobic environments	83
3.3.3.3.1.1. Comparing of fitness responses of lineages adapted to narrow or broad niches.....	84
3.4. Summary.....	87
Chapter Four : Genotypic analysis of lineages adapting to aerobic and anaerobic environments ..	89
4.1. Introduction.....	89
4.2. Objectives.....	90
4.3. Results and discussion.....	90
4.3.1. Observation of polymorphism among evolved lineages.....	90
4.3.2. Modification of the ancestral genome sequence.....	91
4.3.3. Mutation analysis.....	93
4.3.3.1. Classification of mutations.....	93
4.3.3.2. Evidence of cross contamination.....	93
4.3.3.3. Critical analysis of mutations.....	96
4.3.3.4. Increased occurrence of IS elements within evolved clones.....	100
4.3.4. Identifying evolutionary pathways within the adaptive landscapes.....	101
4.3.4.1. Mechanisms of adaptation to different treatments.....	103
4.3.4.1.1. Modification of anaerobic fermentation pathways.....	103
4.3.4.1.1.1. Mutations in <i>nadR</i>	105
4.3.4.1.1.2. Mutations in <i>pflB</i>	106
4.3.4.1.1.3. Re-activation of <i>dcuS</i>	106
4.3.4.1.1.4. Mutations in <i>adhE</i>	107
4.3.4.1.2. Modification of the toxin-antitoxin systems.....	108
4.3.4.1.2.1. Mutations in the <i>hokC/nhaA</i> locus.....	110
4.3.4.1.2.2. Mutations in the <i>trg/mokB</i> locus.....	111
4.3.4.1.2.3. Mutations in the <i>ECB_01533/hokD</i> locus.....	112
4.3.4.1.2.4. Mutation in the <i>insA-7/hokE</i> locus.....	112
4.3.4.1.2.5. Mutations in the <i>ldr</i> locus.....	113
4.3.4.1.2.6. Adaptation through mutation of toxin and antitoxin systems.....	114
4.3.4.1.3. Deletions of cryptic prophages.....	114

4.3.4.1.3.1. Deletion of cryptic P22 prophage	116
4.3.4.1.3.2. Deletion of cryptic 186 prophage	118
4.3.4.1.3.3. Deletion of cryptic Qin prophage.....	118
4.3.4.1.3.4. Deletion of cryptic P2 prophage	118
4.3.4.1.3.5. Adaptation through prophage excisions.....	119
4.3.4.1.4. Inactivation of virulence determining genes	120
4.3.4.1.4.1. Mutations in <i>agn43</i>	121
4.3.4.1.4.2. Mutations in the <i>kps</i> cluster	122
4.3.4.1.4.3. Adaptation through loss of function of virulence gene	123
4.3.4.1.5. Mutations of the <i>brnQ</i> gene	123
4.3.4.1.6. Mutations of the <i>cycA</i> gene	124
4.3.4.1.7. Mutations of <i>rpo</i> genes.....	125
4.3.4.1.7.1. Adaptation through GASP mutations	125
4.3.4.1.8. Mutations of the <i>pcnB</i> gene	126
4.3.5. Exploring the adaptive landscapes	127
4.3.5.1 Evolutionary pathways undertaken by aerobic lineages	127
4.3.5.2 Evolutionary pathways undertaken by anaerobic lineages	130
4.3.5.3 Evolutionary pathways undertaken by fluctuating lineages.....	130
4.4. Summary	132

Chapter Five : Investigating the origin and maintenance of diversity in the anaerobic lineages...135

5.1. Introduction	135
5.2. Objectives.....	136
5.3. Results and discussion	136
5.3.1. SCM within anaerobic lineages.....	136
5.3.2. Genetic basis for SCM in the anaerobic environment	138
5.3.2.1. Colony polymorphism due to multiple mutations	140
5.3.2.2. Colony polymorphism due to $\Delta insB-6-ybdK$	142
5.3.3. Evolutionary dynamics of polymorphic populations within the anaerobic environment	143
5.3.3.1. Existence of a stable equilibrium	143
5.3.3.2. Evidence of cross-feeding	145
5.3.3.3. Relative fitness of AN7	147

5.3.4. Fixation of a <i>glgC</i> mutation in anaerobic lineages	150
5.3.4.1. Mutation in <i>glgC</i>	150
5.3.4.1.1. Characterisation of the <i>glgC</i> mutation	151
5.3.4.1.2. Implications of genetic background on <i>glgC</i> mediated inhibition	152
5.3.4.1.3. Implications of growth conditions on <i>glgC</i> mediated inhibition	153
5.3.4.1.3.1. Potential non-contact inhibition of REL4536 by AN7	154
5.3.5. Loss of fitness within AN7.....	154
5.3.5.1. Loss of <i>appY</i>	155
5.3.5.2. Partial deletion in <i>arcB</i>	158
5.3.5.2.1. Effect of deleterious mutations in AN-1K-7	159
5.3.5.3. A model for the evolution of the AN7 lineage	160
5.4. Summary.....	162
Chapter Six : Final discussion	165
6.1 Further discussion and conclusions.....	165
6.2 Future perspectives	168
6.3 Summary.....	170
Chapter Seven : Appendix	173
Chapter Eight : References	302

List of figures

Figure 1.1	Comparison of respiration in aerobic environments and fermentation in anaerobic environments in <i>E. coli</i>	4
Figure 1.2	Enzymes involved in anaerobic fermentation	5
Figure 1.3	The ArcBA and Fnr aerobic/anaerobic response reactions	7
Figure 1.4	Wright's adaptive landscape	15
Figure 1.5	The actualisation step of the cit ⁺ phenotype in the Ara-3 population	24
Figure 1.6	Phenotypic diversity among <i>P. fluorescens</i> SBW25 populations	28
Figure 1.7	Diagrammatic overview of this thesis	34
Figure 2.1	Overview of lineage establishment at Day 0	49
Figure 2.2	Set up of 24-well plate	50
Figure 3.1	Growth curves of <i>E. coli</i> REL4536 in aerobic and anaerobic conditions	65
Figure 3.2	Growth curves of <i>E. coli</i> REL4536 in DM0 and DM25 in aerobic and anaerobic conditions	67
Figure 3.3	Cell densities in all treatments over 4,000 generations	70
Figure 3.4	Average relative fitness of aerobic lineages over 4,000 generations	76
Figure 3.5	Relative fitness trajectories of individual aerobic lineages over 4,000 generations	78
Figure 3.6	Average relative fitness of anaerobic lineages over 4,000 generations	80
Figure 3.7	Relative fitness trajectories of anaerobic lineages over 4,000 generations	82
Figure 3.8	Average fitness of fluctuating lineages over 4,000 generations	84
Figure 3.9	Generalist adaptation of fluctuating lineages as compared to aerobic and anaerobic lineages at 2,000 generations	85
Figure 4.1	Flow chart for identification of likely adaptive mutations	97
Figure 4.2	Venn diagram of genes and operons with putative adaptive mutations that arose during evolution under the aerobic, anaerobic and fluctuating treatments	102
Figure 4.3	Diagram of the anaerobic fermentation pathways in <i>E. coli</i>	104
Figure 4.4	Location of <i>hok/sok</i> and <i>ldr</i> toxin-antitoxin system genes within the <i>E. coli</i> REL453 genome	109

Figure 4.5	Mutation events located between <i>hokC</i> and <i>nhaA</i> genes in evolved lineages	111
Figure 4.6	Mutation events located between the <i>trg</i> and <i>mokB</i> genes in evolved lineages	112
Figure 4.7	Mutation events located between the <i>ECB_01533</i> and <i>hokD</i> genes in evolved lineages	112
Figure 4.8	Mutation event located between the <i>insA-7</i> and <i>hokE</i> genes in evolved lineages	113
Figure 4.9	Mutation events located near the <i>ldr</i> gene clusters in <i>E. coli</i> REL4536	113
Figure 4.10	Locations of nine cryptic prophages within the <i>E. coli</i> REL4536 genome	115
Figure 4.11	The <i>kps/kfi</i> operon	122
Figure 5.1	Agar plate containing typical and TCM and SCM morphotypes	137
Figure 5.2	Venn diagram of collective mutations in each of the three colony morphotype groups (SCM Type 1, SCM Type 2, and TCM) from 2,000 generation anaerobically evolved clones	139
Figure 5.3	Investigation of co-existence between SCM and TCM	144
Figure 5.4	Cross-feeding between TCM- and SCM-treated cultures for the three populations in which SCM clones were isolated	145
Figure 5.5	Relative fitness of AN7 clones over 4,000 generations	148
Figure 5.6	Representation of population morphotype frequency, relative fitness, mutations and average cell densities in anaerobically evolving lineages over 4,000 generations	149
Figure 5.7	Glycogen synthesis pathway	151
Figure 5.8	The mutations in the <i>glgC</i> gene as reported in this thesis and by Lemonnier <i>et al.</i> 2008	152
Figure 5.9	The domain structure of ArcB	158
Figure 5.10	Proposed evolution of the AN7 lineage	161

List of tables

Table 1.1	Summary of competitive fitness data from LTEE studies with <i>E. coli</i> B reporting adaptive mutations	21
Table 2.1	Bacterial strains used in this study	39
Table 2.2	Antibiotics used in this study	46
Table 2.3	PCR reaction composition for PCR using Platinum® Taq	56
Table 2.4	Sample and library construction details for the genomes sequenced in this study	59
Table 2.5	Bioinformatic resources and software used in this study	61
Table 3.1	Relative fitness of the six Ara ⁺ mutants compared to REL4536 under aerobic conditions	71
Table 3.2	Relative fitness of three Ara ⁺ mutants compared to REL4536 under anaerobic conditions	72
Table 3.3	Aerobic and anaerobic competitive fitness assay results for nalidixic acid resistant mutants and rifampicin resistant mutants	74
Table 4.1	Mutations detected from the genome re-sequencing of REL4536 as compared to the REL4536 genome sequence	91
Table 4.2	Mutation types and classes as reported within all 42 evolved clones when compared to the ancestral <i>E. coli</i> REL4536 strain	93
Table 4.3	Number of shared mutations	94
Table 4.4	The IS elements in the <i>E. coli</i> REL4536 genome	100
Table 4.5	Average number of IS element insertion of IS1, IS150 and IS186 mutations per clone in all treatments at 2,000 generations	101
Table 4.6	Genes mutated in anaerobic fermentation pathways	104
Table 4.7	Mutations in TA systems within evolved lineages	109
Table 4.8	List of prophage excisions reported in this study	115
Table 4.9	Inactivation of virulence genes	120
Table 4.10	Modification of the <i>brnQ</i> gene	124
Table 4.11	Putative adaptive traits occurring in lineages at 2,000 generation during LTEE	128

Table 5.1	Population compositions of anaerobic lineages throughout 4,000 generations	138
Table 5.2	List of mutations in AN-1K-7	155
Table 5.3	Table of genes deleted in the <i>ΔinsB-6-ybdK</i> deletion event in AN-1K-7	156
Table 7.1	List of primers used in this study	173
Table 7.2	Raw genome sequence data	174
Table 7.3	List of all mutation in aerobically evolved genomes	175
Table 7.4	List of all mutation in anaerobically evolved genomes	190
Table 7.5	List of all mutation in fluctuating genomes	237
Table 7.6	List of all mutations arising in the aerobic environment	285
Table 7.7	List of all mutations arising in the anaerobic environment	286
Table 7.8	List of all mutations arising in the fluctuating environment	289
Table 7.9	List of all common mutations arising in the more than one environment	292
Table 7.10	Synonymous SNP mutations arising in different treatments and generations	293
Table 7.11	Evidence of identical mutations in the 4,000 generation anaerobic lineages	294
Table 7.12	Online mutations between 2,000 and 4,000 genomic data	296
Table 7.13	List of genes or operons that have acquired multiple mutations among lineages in the three conditions of study	297

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
<i>g</i>	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	Phosphoenolpyruvate
ppGpp	Guanosine pentaphosphate
Rif ^r	Rifampicin resistant reference strain
RNA	Ribonucleic 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
