

# Coupling Stress Responses and Growth Pathways in *Haemophilus influenzae*

Changde Donald Jiang, B. Biotech. (Honours)



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## ABSTRACT

*Haemophilus influenzae* is an obligate human pathogen which requires NAD and heme to grow. Commonly a commensal within the nasopharynx, *H. influenzae* can be found in both encapsulated and non-encapsulated forms. Encapsulated species, primarily the serotype type b, can be invasive and can cause bacteraemia while the non-encapsulated forms, otherwise known as non-typeable *H. influenzae* (NTHi), infect the upper respiratory tract, and occasionally causing the recurrence of chronic airway diseases. *H. influenzae* requires different pathways for bacterial growth as it can move to and cause diseases within diverse sites of the body where the physical and chemical properties are different. The bacteria encounter different reactive chemical stresses that may be produced endogenously (through the bacterial metabolic pathways) and exogenously (host-generated chemicals) within the different niches. The presence and nature of these reactive chemical stresses impact on their ability to survive, their pathogenesis and the disease outcome within the niches. This therefore highlights a link between the capacity of the bacteria to successfully respond to the environmental stresses and their selection of their own metabolic pathways for energy, biosynthesis and intracellular control of redox balance. In addition, the response to a particular host environment is not only dependent on these stresses, but also on other molecules or biochemicals present in the local environment and the interplay between these factors will highly impact on the bacteria's colonisation and survival. This interplay is poorly understood and is central to the work presented here.

A key example of such interplay is the AdhC system. This thesis represents the work done to characterise AdhC and determine its role in the metabolism of *H. influenzae* and its links to exogenous stress response and the environmental conditions. The *adhC* gene from *H. influenzae* encodes the glutathione-formaldehyde dehydrogenase AdhC which has been found to be essential for defence against the host-generated chemical S-nitrosoglutathione (GSNO; the product of reactive nitric oxide combining with glutathione). Studies have further suggested

that AdhC might be required for the growth of the bacteria with oxygen, glucose and iron. *adhC* and *estD* are regulated by the redox sensitive NmlR but given the link to these other environmental factors, there is some indication that this pathway is coupled to the global regulators that sense oxygen, glucose and iron.

It was aimed to investigate the stress response regulation connected to environmental oxygen and carbon metabolism in *H. influenzae*. One of the major environmental stresses in normal growth is the generation of reactive oxygen species (ROS) in the presence of oxygen and FNR is one of the global regulators that regulate genes in response to oxygen changes. Our results showed that FNR regulates at least 279 genes in high and low oxygen tensions. Our results also implied that another global regulator, CRP, is required for utilising mannose and glucose, or the pathways for their transport and metabolism.

*H. influenzae* has been shown to colonise anatomical niches that possess non-optimal conditions by producing biofilms. Therefore, another aspect of this work was to determine the impact of stress responses to biofilm formation in *H. influenzae*. Our results showed that more biofilms were produced in *adhC*, *nmlR*, *fnr* and *crp* mutants than in Rd KW20, under both normal aerobic conditions and stressed conditions, suggesting that these genes are essential for normal aerobic growth as well as stress responses. RNA sequencing was further performed on a known biofilm-forming *nikQ* mutants in order to gain a deeper insight into *H. influenzae*'s ability to form biofilms. Significant changes in the surface structures in this lifestyle state were found.

## TABLE OF CONTENTS

Declaration by author	...i
Acknowledgements	...iii
Abstract	...v
Table of Contents	...vii
List of figures	...xiii
List of tables	...xvii
List of abbreviations used in this thesis	...xix
<b>Chapter 1: Introduction and Literature Review</b>	<b>...1</b>
1.1 Introduction	...2
1.2 <i>Haemophilus influenzae</i>	...4
1.2.1 Background	...4
1.2.2 Non-typeable <i>Haemophilus influenzae</i> (NTHi)	...6
1.3 Host Defences	...7
1.3.1 Nitric oxide and reactive oxygen species	...7
1.3.2 Reactive aldehydes	...8
1.3.3 Iron	...10
1.3.4 GSNO and AdhC	...14
1.4 Gene regulation in response to host-derived stress	...15
1.4.1 Transcriptional response to oxidative stress: MerR family	...15
1.4.2 NmlR	...18
1.4.3 Global regulators and stress response	...21
1.4.3.1 Cpx	...21
1.4.3.2 CRP-FNR superfamily	...22

1.4.4	Biofilm formation	...25
1.5	The Project	...28
1.5.1	Previous research	...28
1.5.2	Significance of project	...29
1.5.3	Aims of project	...30
<b>Chapter 2: Materials and Methods</b>		<b>...33</b>
2.1	Media Composition	...34
2.1.1	Luria-Bertani broth (LB)	...34
2.1.2	Brain Heart infusion media (BHI media)	...34
2.1.3	Chemically defined Media (CDM)	...34
2.2	Bacterial strains and growth conditions	...35
2.3	Bioinformatics	...36
2.4	PCR	...37
2.4.1	PCR primers	...37
2.4.2	PCR amplification protocol	...38
2.4.3	Splice overlapping PCR	...39
2.5	General molecular techniques	...40
2.5.1	Gel extraction and PCR purification	...40
2.5.2	Miniprep of plasmid extraction	...40
2.5.3	Restriction endonuclease digestion	...40
2.5.4	DNA modification and ligation	...40
2.6	Transformation of cells	...41
2.6.1	Transformation into <i>Escherichia coli</i> DH5 $\alpha$	...41
2.6.2	Transformation into <i>Haemophilus influenzae</i>	...41
2.7	Bacterial growth assays	...42
2.7.1	Oxygen tension in flasks	...42
2.7.2	Oxygen tension in 96-wells plates	...42
2.7.3	Growth with stressors	...42

2.7.4	Growth for iron requirement	...43
2.7.5	Growth with different carbon sources	...43
2.8	Aggregation assays	...44
2.9	Biofilm assays	...44
2.10	RNA sequencing	...44
2.10.1	Cell harvesting	...44
2.10.2	RNA extraction	...45
2.10.3	mRNA enrichment	...45
2.10.4	RNA sequencing with Illumina	...46
2.10.5	Ion torrent RNA sequencing	...46
2.10.6	Data analysis	...47
<b>Chapter 3: Characterisation of <i>Haemophilus influenzae</i> Rd KW20 <i>adhC</i></b>		<b>...49</b>
3.1	Introduction	...50
3.2	<i>In silico</i> analysis of <i>nmlR-adhC</i> in <i>H. influenzae</i>	...51
3.3	AdhC and NmlR are essential for growth under high oxygen tension	...54
3.4	AdhC is essential for protection against formaldehyde	...56
3.5	AdhC is essential for detoxification of glycolaldehyde	...58
3.6	AdhC has a role in excess of iron	...59
3.7	AdhC is required to utilize galactose and fucose as carbon sources	...62
3.8	EstD is essential for growth in high oxygen tension	...65
3.9	AdhC is not involved in superoxide detoxification processes	...66
3.10	Discussion	...67
<b>Chapter 4: <i>H. influenzae</i> and Oxygen: Transcriptomics on Oxygen Variations</b>		<b>...73</b>
4.1	Introduction	...74
4.2	Differentially expressed genes	...76
4.3	Identification of potential small RNAs	...80

4.4	Discussion	...84
Chapter 5: Physiology of non-typeable <i>Haemophilus influenzae</i> 86-028NP		...91
5.1	Introduction	...92
5.2	<i>adhC</i> is a pseudogene in 86-028NP	...93
5.3	86-028NP grew poorly under low oxygen	...94
5.4	86-028NP is sensitive to formaldehyde and glycolaldehyde	...96
5.5	86-028NP has attenuated growth in the absence of iron	...98
5.6	86-028NP has a different growth pathway	...100
5.7	Differentially expressed genes in 86-028NP under high oxygen tension	...103
5.8	Discussion	...108
Chapter 6: Regulation connected to oxygen levels and carbon metabolism in <i>Haemophilus influenzae</i>		...113
6.1	Introduction	...114
6.2	Construction of <i>crp</i> and <i>cpx</i> mutant strains in Rd KW20	...115
6.3	CRP and its link to growth with carbon sources	...116
6.4	<i>fnr</i> mutants in Rd KW20 have low growth in high and low oxygen tension	...117
6.5	Transcriptomics by RNA sequencing on the <i>fnr</i> mutants in Rd KW20	...121
6.6	Discussion	...145
Chapter 7: The role of oxygen linked pathways for <i>Haemophilus influenzae</i> survival mechanisms in the host		...151
7.1	Introduction	...152
7.2	<i>adhC</i> mutants form cell aggregates under GSNO stress, but not under formaldehyde and hydrogen peroxide stresses	...153
7.3	86-028NP and <i>fnr</i> mutants form cell aggregates	...154

7.4	Cell aggregates formation increases with decreasing oxygen tension in chemically defined media	...156
7.5	<i>H. influenzae</i> forms biofilm within 24 hours and biofilm increases with time	...157
7.6	Mutants form much biofilm with and without GSNO	...159
7.7	<i>adhC</i> and <i>nmlR</i> mutants form much less biofilm in presence of formaldehyde	...161
7.8	<i>adhC</i> mutants produce biofilm in presence of glucose and ribose in high oxygen tension and in presence of fructose in low oxygen tension	...163
7.9	RNA sequencing on <i>nikQ</i> mutants	...165
7.10	Discussion	...168
Chapter 8: Conclusion		...177
References		...181
Appendices		...207
Supplementary files		...S1



## LIST OF FIGURES

Figure 1.1: ROS and RNS production in mammalian cells.....	8
Figure 1.2: Oxidation of sugars to $\alpha,\beta$ -dicarbonyl compounds.....	10
Figure 1.3: Fenton Chemistry.....	11
Figure 1.4: The model for MerR action showing the distortion of the <i>mer</i> operator/promoter.....	16
Figure 1.5: Structure of the promoter/ operator region of the <i>mer</i> operon of Tn501.....	17
Figure 1.6: Model of biofilm development.....	25
Figure 3.1: Graphical gene arrangement of <i>nmlR/ adhC</i> .....	51
Figure 3.2: Alignment of NmlR.....	52
Figure 3.3: Alignment of intergenic spacer region.....	52
Figure 3.4: Alignment of AdhC.....	53
Figure 3.5: Growth profiles of Rd KW20 and <i>adhC</i> mutants at different oxygen tensions.....	55
Figure 3.6: Growth profiles of Rd KW20 and <i>nmlR</i> mutants at different oxygen tensions.....	55
Figure 3.7: Growth profiles of Rd KW20 and <i>adhC</i> mutants in presence of formaldehyde.....	57
Figure 3.8: Growth profiles of Rd KW20 and <i>adhC</i> mutants in presence of glycolaldehyde under high oxygen tension.....	58
Figure 3.9: Growth profiles of Rd KW20 and <i>adhC</i> mutants in iron depleting environment.....	61
Figure 3.10: Growth profiles of Rd KW20 and <i>adhC</i> mutants with different carbon sources in high oxygen tension.....	63

Figure 3.11: Growth profiles of Rd KW20 and <i>adhC</i> mutants with different carbon sources in low oxygen tension.....	64
Figure 3.12: Growth profiles of Rd KW20 and <i>estD</i> mutants at different oxygen tensions.....	65
Figure 3.13: Growth profiles of Rd KW20 and <i>adhC</i> mutants in presence of menadione.....	67
Figure 4.1: Flow diagram of the steps involved in typical microbial transcriptome sequencing.....	75
Figure 5.1: Graphical gene arrangement of <i>nmlR/ adhC</i> in 86-028NP.....	93
Figure 5.2: ClustalW alignment of <i>adhC</i> between Rd KW20 and 86-028NP.....	94
Figure 5.3: Growth of 86-028NP under different oxygen tensions.....	95
Figure 5.4: Growth profiles of Rd KW20 and 86-028NP in presence of formaldehyde.....	97
Figure 5.5: Growth profiles of Rd KW20 and 86-028NP in presence of glycolaldehyde.....	97
Figure 5.6: Growth profiles of Rd KW20 and 86-028NP in iron depleting environment.....	99
Figure 5.7: Growth profiles of Rd KW20 and 86-028NP with different carbon sources in high oxygen tension.....	101
Figure 5.8: Growth profiles of Rd KW20 and 86-028NP with different carbon sources in low oxygen tension.....	102
Figure 6.1: Growth profiles of Rd KW20 and <i>crp</i> mutants with different carbon sources in high oxygen tension.....	118
Figure 6.2: Growth profiles of Rd KW20 and <i>crp</i> mutants with different carbon sources in low oxygen tension.....	119
Figure 6.3: Growth profiles of Rd KW20 and <i>fnr</i> mutants at different oxygen tensions.....	120

Figure 6.4: An outline of the expected changes in gene expression observed from the transcriptomics of Rd KW20 and its isogenic <i>fnr</i> mutant.....	131
Figure 7.1: Aggregation of <i>H. influenzae</i> Rd KW20, 86-028NP and <i>adhC</i> mutants in presence of stressors.....	154
Figure 7.2: Aggregation of <i>H. influenzae</i> Rd KW20, 86028NP, <i>crp</i> mutants and <i>fnr</i> mutants.....	155
Figure 7.3: Aggregation of <i>H. influenzae</i> Rd KW20 and <i>fnr</i> mutants in different oxygen tensions.....	157
Figure 7.4: Biofilm formation of <i>H. influenzae</i> .....	158
Figure 7.5: Microtitre plate biofilm formation of Rd KW20, 86-028NP and mutants in presence of GSNO.....	160
Figure 7.6: Microtitre plate biofilm formation of Rd KW20, 86-028NP and mutants in presence of formaldehyde.....	162
Figure 7.7: Microtitre plate biofilm formation of Rd KW20, 86-028NP and mutants with different carbon sources.....	164



## LIST OF TABLES

Table 1.1: Comparison of encapsulated (type b) and non-encapsulated (non typeable) <i>H. influenzae</i> .....	5
Table 1.2: CRP regulation in bacteria.....	23
Table 1.3: Examples of biofilm related proteins.....	27
Table 2.1: Modification of chemically defined media.....	35
Table 2.2: Bacterial strains, plasmids and antibiotics used.....	35
Table 2.3: Primers used in chapter 3.....	37
Table 2.4: Primers used in chapter 6.....	38
Table 4.1: Rd KW20 genes differentially expressed between high and low oxygen.....	78
Table 4.2: List of potential sRNAs in high oxygen tension.....	81
Table 4.3: List of potential sRNAs in low oxygen tension.....	82
Table 4.4: Unique potential small RNAs in high oxygen tension.....	83
Table 4.5: Unique potential small RNAs in low oxygen tension.....	84
Table 5.1: 86-028NP genes differentially expressed between high and low oxygen.....	105
Table 5.2: Upregulated genes in 86-028NP against Rd KW20 in high aerobic condition.....	106
Table 5.3: Downregulated genes in 86-028NP against Rd KW20 in high aerobic condition.....	107
Table 6.1: Rd KW20 <i>fnr</i> mutants genes differentially expressed between high and low oxygen.....	123
Table 6.2: Genes differentially expressed in Rd KW20 compared to Rd KW20 <i>fnr</i> mutants under high oxygen.....	126

Table 6.3: Genes differentially expressed in Rd KW20 compared to Rd KW20 <i>fnr</i> mutants under high oxygen.....	129
Table 6.4: Genes differentially expressed between Rd KW20 and <i>fnr</i> mutants at high oxygen.....	132
Table 6.5: Genes differentially expressed between Rd KW20 and its <i>fnr</i> mutants under high oxygen and further changed in the <i>fnr</i> mutants with low oxygen.....	135
Table 6.6: Genes differentially expressed between Rd KW20 <i>fnr</i> high and low oxygen condition.....	136
Table 6.7: Genes differentially expressed between Rd KW20 under high and low oxygen as well as changed in <i>fnr</i> mutant sin low oxygen condition.....	137
Table 6.8: Genes differentially expressed between Rd KW20 and its <i>fnr</i> mutants under low oxygen condition.....	138
Table 6.9: Genes differentially expressed between Rd KW20 high and low oxygen conditions and further changed in the <i>fnr</i> mutants in high oxygen.....	142
Table 6.10: List of FNR regulated genes (from the Virtual Footprint <i>in silico</i> analysis).....	143
Table 7.1: Genes differentially expressed in Rd KW20 compared to Rd KW20 <i>nikQ</i> mutants.....	167

**LIST OF ABBREVIATIONS USED IN THIS THESIS**

$\Delta$	Mutant
A630	Absorbance at 630nm
ABC	ATP-binding cassette
Amp <sup>r</sup>	Ampicillin resistant
ATP	Adenosine triphosphate
B	Billion
BHI	Heart Infusion
bp	base pairs
cAMP	cyclic adenosine monophosphate
CDM	Chemically Defined Media
cDNA	complementary DNA
CIP	Calf intestinal alkaline phosphatase
CoA	Coenzyme A
COPD	Chronic obstructive pulmonary disease
DAB	Diaminobenzene
DAM	Deferoxamine mesylate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide

DJ

List of Abbreviations

EP End point

FDH Formaldehyde

Fe Iron

Fru Fructose

Fuc Fucose

Gal Galactose

Gbp Giga base pairs

Glu Glucose

GO Gene ontology

GSH Glutathione

GSH-FDH Glutathione-dependent formaldehyde dehydrogenases

GSNO S-nitrosoglutathione

GSSG Glutathione disulphide

h Hour(s)

HNE 4-hydroxynonenal

HO• Hydroxyl radicals

HOCl Hypochlorous acid

HOONO Peroxynitrous acid

IGS Intergenic spacer region

iNOS Inducible nitric oxide synthase

Kan <sup>r</sup>	Kanamycin resistant
kb	Kilobases
LB	Luria-Bertani
LOS	Lipooligosaccharides
Man	Mannose
Mbp	Mega base pairs
MDA	Malondialdehyde
min	minutes
mRNA	Messenger RNA
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NaPy	Sodium pyruvate
NCBI	National Center for Biotechnology Information
NO	Nitric oxide
NTHi	Non-typeable <i>H. influenzae</i>
O/P	Operator/ promoter
OD	Optical density
OM	Otitis media
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction

DJ	
PPIX	Protoporphyrin IX
RA	Reactive aldehydes
Rib	Ribose
RIN	RNA integrity number
RNA	Ribonucleic acid
RNAP	RNA polymerase
RNS	Reactive nitrogen species
RO•	Alkoxy radicals
ROO•	Peroxy radicals
ROS	Reactive oxygen species
RPKM	Reads per kilo base per million
rpm	revolutions per minute
rRNA	Ribosomal RNA
RSNO	S-nitrosothiols
sRNA	Small RNA
TCA	Tricarboxylic acid cycle
TE	Tris EDTA
U	Units
w/v	weight/ volume
WT	Wild type