Coupling Stress Responses and Growth Pathways in *Haemophilus influenzae*

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THE UNIVERSITY of ADELAIDE

Submitted for the degree of Doctor of Philosophy

Discipline of Microbiology and Immunology

The School of Molecular and Biomedical Science

The University of Adelaide

June 2013

DECLARATION

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Adelaide, Australia, June 2013

Changde Donald Jiang

ACKNOWLEDGEMENTS

My heartfelt thanks to Dr Stephen Kidd for giving me a chance to do my PhD with him. This thesis would have been impossible if not for his guidance, advice, patience and support for the past 4 years. A big gratitude to Dr Stephen Bent for the assistance in analysing my RNA data. I would not have done that without you. Thanks also to Long and Alex for making my days in the lab less boring.

Big thanks to the Morona's and Paton's lab, with mentions to Adam, Alistair, Claudia, Layla, Richard, Brooke, Liz, Catherine, Steph and Lauren. I have already forgotten how many "n" times have I "borrowed" stuff from you guys, and I will never forget the kindness. Without you guys, I would have not been able to accomplish my experiments and ultimately, my PhD. Kudos to McDevitt's lab, especially to Victoria and Miranda, for making the lab so much livelier. I apologise for missing you guys out from my presentation slides.

Thanks to all those I have come across during the past 4 years. Thanks to my fellow PhD friends, namely Minyan, Ah Lum, Titi, Zarina, "Radish" and Jenni. You guys are often my answers to questions and I do look up to you when I am in trouble. Special thanks to Chris for giving me a chance to demonstrate and Anita for being so patient and generous towards me. There are still so many more people I would love to mention but could not. Do believe me when I say you guys are in my heart.

I would also like to thank my family for their support and annual visits. Without them, I would not have reached where I am today. Finally, many thanks to all my friends in Australia and Singapore, especially to Masa, Angela, Janet, Wanting, Waichee, Muqi, Beeling, Zach and Ivy. You guys have made my stay in Adelaide so much more fun and less lonely. And finally to Calvin

and Felix for making my days back in Singapore more enjoyable.

Lastly, I would like to acknowledge University of Adelaide and Australia Commonwealth Government for the provision of my scholarship and everyone who have assisted in putting this thesis together.

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-genearted 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*-nitrosogluthatione (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 regulatos 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.

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LIST OF ABBREVIATIONS USED IN THIS THESIS

Δ	Mutant
A630	Absorbance at 630nm
ABC	ATP-binding cassette
Amp^r	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

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^r	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 *H. influenzae*
O/P	Operator/ promoter
OD	Optical density
OM	Otitis media
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction

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