Haemophilus influenzae survival and biofilm formation in a complex physical, chemical and multi-species environment

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Table of Contents

Declaration

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Alexandra Tikhomirova,

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Abstract

H. influenzae is an opportunistic human pathogen capable of occupying a range of niches in the respiratory tract. Both during health and disease processes, *H. influenzae* must adapt to the conditions present in the microniches it encounters and correspondingly alter its lifestyle in the presence of non-optimal conditions. The niches encountered by *H. influenzae* in the human host include the presence of chemical stress such as ROS and RCS compounds, as well as a diverse range of pH conditions ranging from pH 7-9, and the presence of other members of the microflora, such as *Streptococcus pneumoniae*.

In this thesis, we have identified that there are strain-specific components related to the adaptation of *H. influenzae* to specific conditions. We have identified that different *H. influenzae* isolates employ different mechanisms to adapt to the presence of diverse, and often stress-inducing conditions. One adaptation mechanisms employed by *H. influenzae* is the adoption of a sessile biofilm lifestyle. We have shown that there is a strain-specific response of *H. influenzae* isolates in their biofilm formation to the presence of different nutrient conditions, and to the presence of ROS and RCS such as formaldehyde, methylglyoxal and H_2O_2. We have also shown that in different nutrient conditions, there is a different requirement for eDNA in the EPS matrix of individual strains.

In addition, we have identified a role of the nickel import system *nikKLMQO-nimR* of *H. influenzae* in its biofilm formation. We have shown that when this system is absent, or when *H. influenzae* is in a nickel limited environment, the *H. influenzae* cells display an increased biofilm formation. This biofilm formation response was accompanied by a global transcriptomics response, which displayed global changes in metabolic pathways.

Further to these findings, we have shown that there are strain-specific differences in *H. influenzae* adaptation to different pH conditions. These differences were expressed both as differences in biofilm formation, and differences on the transcriptomics level.

Another significant finding was that the pH played an important role in the inter-species interactions of *H. influenzae* and *S. pneumoniae*. In a batch culture system in stationary phase at a lower initial pH of 7.4, *S. pneumoniae* was able to convert *H. influenzae* into a VBNC

state. However, *H. influenzae* was able to survive in a culturable state in co-culture in log phase, or when a higher initital pH was used. We have also shown that in co-culture, there were significant transcriptomics changes both in *H. influenzae* and *S. pneumoniae*, including an induction of stress response genes in *H. influenzae*, and a down-regulation of sugar utilisation genes in *S. pneumoniae*.

Importantly, we have shown that in a continuous flow cell system, *H. influenzae* and *S. pneumoniae* exist in a different lifestyle and different transcriptomics profile than in a batch culture system, both in mono- and co-culture. In this system, the 2 species were able to co-exist without the reduction in culturability in either of the species. We have also shown that the transcriptomic profile in co-culture in a flow cell system is different to what was observed in the batch system, with one of the major findings being the up-regulation of sugar utilisation genes in *S. pneumoniae*, suggesting the potential metabolic relationship between *H. influenzae* and *S. pneumoniae*.

We have investigated this finding further, and have indeed demonstrated that nutrient availability and carbon source impact the *H. influenzae/ S. pneumoniae* interactions. In a flow cell system containing a nutrient-limited CDM media with glucose, *H. influenzae* was able to survive equally in mono- and co-culture. However, *S. pneumoniae* was unable to grow in mono-culture, and in co-culture displayed 3 phenotypes: a wild-type phenotype at 24 h, an undetectable state until 336 h, and a small colony variant state at 336 h. *S. pneumoniae* did not significantly impact the *H. influenzae* transcriptome in co-culture in either the undetected or SCV state, but did subtly modify the *H. influenzae* transcriptome upon transition to different time-points of growth, or different nutrient conditions. We have also preliminarily identified transcriptomic changes in *S. pneumoniae* at 64 h and 336 h, which correspond to a persister-cell like state observed in other bacterial species.

Overall, we have identified that environmental factors significantly impact the ability of *H. influenzae* to survive and to adopt lifestyles pertaining to these environments, in a strain-specific manner. We have shown that these adaptations are often accompanied by global transcriptomics changes. We have also identified that the inter-species interactions between *H. influenzae* and *S. pneumoniae* are highly complex and their outcome depends on a multitude of environmental conditions.

List of Publications

The work presented in this thesis has contributed to a range of publications listed below.

- **Tikhomirova A**, Kidd SP. The outcome of *Haemophilus influenzae* and *Streptococcus pneumoniae* inter-species interactions depends on pH, nutrient availability and growth phase. *International Journal of Medical Microbiology*. *In Press.*

- **Tikhomirova A**, Jiang D, Kidd SP. A new insight into the intracellular nickel levels for the stress response, surface properties and twitching motility by *Haemophilus influenzae*. 2015. *Metallomics*. 8:650-61.

- Ishak N, **Tikhomirova A**, Bent SJ, Ehrlich GD, Hu FZ, Kidd SP. There is a specific response to pH by isolates of *Haemophilus influenzae* and this has a direct influence on biofilm formation. 2014. *BMC Microbiology*. 14:47

- **Tikhomirova A**, Kidd SP. *Haemophilus influenzae* and *Streptococcus pneumoniae*: living together in a biofilm. 2013. *Pathogens and Disease*. 69:114-26

List of Abbreviations

CDM	chemically defined medium
CFU	colony forming unit
COM	chronic otitis media
COPD	chronic obstructive pulmonary disease
CV	crystal violet
DNA	deoxyribonucleic acid
eDNA	extracellular DNA
EPS	extracellular polymeric substance matrix
h	hour(s)
HI	heart infusion
H_2O_2	hydrogen peroxide
LB	Luria-Bertani
LOS	lipooligosaccharide
min	minute(s)
ml; µl	milliliter; microlitre
mRNA	messenger RNA
NET	neutrophil extracellular trap
NTHi	non-typeable *Haemophilus influenzae*
OD	optical density
OM	otitis media
PCR	polymerase chain reaction
RCS	Reactive Carbonyl Species

RNA	ribonucleic acid
ROS	Reactive Oxygen Species
RNASeq	RNA sequencing
rpm	revoloutions per minute
SCVs	small colony variants
SEM	scanning electron microscopy
Tfp	type IV (four) pili
VBNC	viable but non-culturable
v/v	volume/volume
WT	wild-type
w/v	weight/volume

List of Figures

Chapter 1

Chapter 3

Chapter 6

Appendix 1

Appendix 2

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 6