Staphylococcus aureus: stress response and its roles in pathogenesis

LONG MINH GIAO BUI

(B.Pharm, M.Sc)

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy from the University of Adelaide

(July, 2015)

Discipline of Microbiology and Immunology

Faculty of Sciences

The School of Biological Sciences

The University of Adelaide

Adelaide, South Australia, Australia

TABLE OF CONTENTS

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to **LONG MINH GIAO BUI** and, to the best of my knowledge and belief, no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

_____

LONG MINH GIAO BUI

Adelaide, July 30th, 2015

ACKNOWLEDGEMENTS

First of all, I am deeply grateful to my supervisor, ***Dr. Stephen Kidd****, for giving me this great opportunity to join the PhD program in the University of Adelaide. His dedicated supervision and support all throughout the tenure of my PhD study helped me to realize the essence of my research. Without his consistent encouragement, flexible scientific approach and useful suggestion this thesis would not be accomplished.*

I would like to sincerely thank my co-supervisors ***Dr. Peter Zilm*** *and* ***Assoc. Prof. Renato Morona*** *for their contributions to direction and richness of this research and great technical support and scholarly advice.*

I am extremely thankful to my lab-mates ***Alexandra Tikhomirova*** *and* ***Donald Jiang*** *for their consistent wonderful help and timely advice during my research pursuit.*

My special thanks also go to all research officers in James Paton's Lab, Renato's Lab and Chris's Lab: ***Dr. Lauren McAllister, Dr. David Ogunniyi, Dr. Layla Mahdi, Dr. Claudia Trapetti, Dr. Adam Potter, Dr. Richard Harvey, Dr. Alistair Standish, Dr. Elizabeth Tran, Dr. Miranda Ween and my lab friends Pratiti Nath, Mabel Lum, Min Teh, Broke Herdman, Zarina Amin, Victoria Lewis and Dr. Peter Cathro*** *who are always very nice when talking to me and giving useful advice as well their experiences.*

I grateful acknowledge the technical collaboration and support from the senior researchers, ***Prof. John Turnidge and Dr. Jan Bell*** *(Adelaide Women's and Children's Hospital) and* ***Assoc. Prof Peter Hoffman*** *(Adelaide Proteomics Centre).*

I extend my deepest thanks to my friends ***Alfred D'sa, Ade Gah, Phuong Nguyen, Dao Huynh, Trung Nguyen, Tran Do*** *and my friends in the RAH for their great mutual friendship, that helped me overcome difficulties with living in Adelaide.*

I would like to acknowledge the great financial support from ***Vietnamese International Education Development*** *and* ***University of Adelaide*** *and the technical support from* ***Discipline of Microbiology and Immunology, University of Adelaide****. My thanks are also for my colleagues of* ***Faculty of Pharmacy, University of Medicine and Pharmacy - Ho Chi Minh City – Vietnam, especially, to Assoc. Prof Dong Tran, Prof. Thanh Tran, Assoc. Prof. Dung Nguyen and Assoc. Prof. Khoi Nguyen*** *for their great support and encouragement.*

Finally, I would like to thank ***my Parents and Sister****, who have scarified everything for me. For their infinite and unconditional care and support, I dedicate the entirety of my work for them.*

……We have to continue to learn. We have to be open. And we have to be ready to release our knowledge in order to come to a higher understanding of reality.

THICH NHAT HANH.

ABSTRACT

Staphylococcus aureus has an incredible ability to survive, either by adapting to environmental conditions or defending against exogenous stresses. In part this ability is provided by the breadth of lifestyles or modes of growth *S. aureus* can adopt. Key to an understanding of chronic, persistent and relapsing *S. aureus* infections is determining the basis for their switch to quasi-dormant lifestyles. Across different bacterial species these alternative lifestyles form a population known as persister cells. It has been proposed that while within their host, a sub-population of *S. aureus* survives in host-generated and therapeutic antimicrobial stress by inducing biofilm growth on host tissue or by growing as Small Colony Variants (SCVs). These stresses include limited nutrition, reactive oxygen species, reactive nitrogen species, other toxic metabolites, cationic peptides, fluctuating pH and osmolarity, several antibiotics, and others that are generated from various host tissues and niches during an infection.

In a multicellular biofilm, the metabolically quiescent bacterial community produces a highly protective extracellular polymeric substance (EPS). The EPS is variously composed of polysaccharides, extracellular DNA (eDNA), and protein, and its protection results in persistent bacterial infections. *S. aureus* forms biofilms in different human tissues, and to some degree the associated EPS has been studied. Alternatively, there exists a diversity of phenotypes and cell-types that translate to a particular bacterial lifestyle. In clinical settings, SCV of *S. aureus* have been observed for many years and when cultured, these cells are non-pigmented colonies *ca.* 10 times smaller than their counterparts on agar plates. Mutations in hemin and menadione biosynthesis (*hemB* and *menA*) have produced laboratory-generated SCVs and these and other mutations have been studied extensively. The presence of gentamicin has also been shown to impede *S. aureus* metabolism and results in SCVs. Both methods result in stable forms of SCV but are artificially generated. Various genotypic factors (single nucleotide polymorphisms, mutations, gene deletions) have been identified to attempt to characterize *S. aureus* SCVs as well as environmental stresses are also considered to be important inducers.

Our project was aimed to investigate the lifestyle switching of *S. aureus* clinical isolates as a stress response to environmental stresses that help these organisms to survive. The results showed that *S. aureus* clinical isolates had relatively similar growth rates but were different in their response to chemical stresses. There were specific strains that responded to stresses by changing their lifestyles to form a biofilm and/or SCVs in harsh conditions (but still sub-lethal levels of stress) but not in lower levels of stress. These results implied that phenotype switching depends on bacterial and host factors and suggests some specific strains may possess a unique pathway involved in surviving when stressed. In addition, studying native characteristics of SCV has been problematic due to their reversion to the parental, rapid growing lifestyle. Using specific host-representative, steady-state growth conditions with low nutrients and growth rates over a prolonged time with methylglyoxal - a naturally resident chemical that is found in the host-pathogen environment, we uniquely induced a *S. aureus* clinical isolate (WCH-SK2) into a stable SCV cell-type. The stable SCV phenotype did not revert after numerous cycles of sub-culturing and analysis revealed it possessed a metabolic and surface profile different from either previously described SCV or biofilm cells.

The existence of the stable SCVs was verified and its features were analyzed by genomic, transcriptomic and surface protein profile studies. Stable SCVs produced an extracellular matrix of protein and extracellular DNA; but not polysaccharide. Compared to its parental cell-type, the stable SCV cells increased expression of certain surface proteins (such as Ebh; host extracellular matrix binding protein homologue) and lantibiotic synthesis while down-regulating factors that stimulate the host immune response (leukocidins, capsule, carotenoids). This cell-type is consistent with a lifestyle protected by a matrix and hidden from immune responses. Genome sequencing revealed a set of genetic changes from the parental to stable SCV cell-type, including the transcription factors RsbB and MrgA, as well as a change in the methylome. Collectively, our data shows that there is heterogeneity within a *S. aureus* population as shown by a diverse scope of cell-types; by growing the cells in conditions that resemble long-term survival in the host, colonization or persistence, we have identified a previously unnoticed *S. aureus* lifestyle. These stable SCV are molecularly distinct in nature to SCV or biofilm cells and this cell-type provides a new understanding of *S. aureus* persistence in the host.

LIST OF PUBLICATIONS

Publications

- Bui, L. M., et al. (2014). "The induction of *Staphylococcus aureus* biofilm formation or Small Colony Variants is a strain-specific response to host-generated chemical stresses." Microbes Infect. **17**(1): 77-82. (Appendix 2)
- Bui, L. M., et al. (2015). "Prolonged Growth of a Clinical *Staphylococcus aureus* Strain Selects for a Stable Small-Colony-Variant Cell Type." Infect Immun. **83**(2): 470-481. (Appendix 3)

Conference Presentations

- Long M.G Bui, Kidd SP. Clinical *Staphylococcus aureus* isolates possess novel lifestyles as a stress response to antimicrobials. ISSSI August 2014 Chicago. Poster presentation
- Long M.G Bui, Kidd SP. Clinical *Staphylococcus aureus* isolates possess novel lifestyles as a stress response. Bacpath Conference August 2013. Poster presentation.
- Long M.G Bui, Kidd SP. Clinical *Staphylococcus aureus* isolates possess novel lifestyles as a stress response. ASM July 2013 – Poster presentation.
- Long M.G Bui, Kidd SP. Clinical *Staphylococcus aureus* isolates possess novel lifestyles as a stress response. BD Student Award Presentation, November-2012.

LIST OF ABBREVIATIONS

AKR	Aldo-Keto Reductase
BA	Blood Agar
BC	Blood Culture
bp	base pair
CDM	Chemically Defined Medium
CA-MRSA	Community Acquired Methicillin Resistant *Staphylococcus aureus*
CFU	Colony Forming Unit
CF	Cystic Fibrosis
DNA	Deoxyribonucleic Acid
GSH	Glutathione
GSNO	S-nitrosoglutathione
HA-MRSA	Hospital Acquired Methicillin Resistant *Staphylococcus aureus*
H_2O_2	Hydrogen Peroxide
h, hr(s)	hour (s)
iNOS	inducible Nitric Oxide Synthase
kb	kilo base
LB	Luria Bertani Broth
LR	Lower Respiratory
min(s)	minute(s)
MG	methylglyoxal
MGE(s)	Mobile Genetic Element(s)
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin Sensitive *Staphylococcus aureus*
NADH	Nicotinamide Adenine Dinucleotide (reduced form)

NADPH	Nicotinamide Adenine Dinucleotide Phosphate (reduced form)
NCBI	National Center for Biotechnology Information
nt	nucleotide
O/N	overnight
OD_{630nm}	Optical density at 630nm
ORF	Open Reading Frame
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PIA	Polysaccharide Intercellular Adhesin
RA	Reactive Aldehydes
RNA	Ribonucleic Acid
RNAP	Ribonucleic Acid Polymerase
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
rpm	rounds per minute
SCVs	Small Colony Variants
SDS	sodium dodecyl sulphate
SEM	Scanning Electron Microscopy
SOD	Superoxide Dismutase
SSTI	Skin and Soft Tissue Infections
TSA	Tryptic Soya Agar
TSB	Tryptic Soya Broth
v/v	volume per volume
VRSA	Vancomycin Resistant *Staphylococcus aureus*
w/v	weight per volume
WHO	World Health Organisation
WT	wild-type

LIST OF FIGURES

LIST OF TABLES