



THE UNIVERSITY
of ADELAIDE

***Staphylococcus aureus: stress response
and its roles in pathogenesis***

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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.

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*.....**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
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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 <i>Staphylococcus aureus</i> |
| CFU | Colony Forming Unit |
| CF | Cystic Fibrosis |
| DNA | Deoxyribonucleic Acid |
| GSH | Glutathione |
| GSNO | S-nitrosoglutathione |
| HA-MRSA | Hospital Acquired Methicillin Resistant <i>Staphylococcus aureus</i> |
| H ₂ O 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 <i>Staphylococcus aureus</i> |
| MSSA | Methicillin Sensitive <i>Staphylococcus aureus</i> |
| 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 <i>Staphylococcus aureus</i> |
| w/v | weight per volume |
| WHO | World Health Organisation |
| WT | wild-type |

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