UNIVERSITY OF TECHNOLOGY SYDNEY

The characterisation of the cell surface of *Staphylococcus aureus* in the search for new therapeutic targets.

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ithreeinstitute

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Kate Harvey, certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except where fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Declaration

All data collected, analysed, and presented in this thesis is the work of the author unless clearly specified. Chapters 2-4 have been prepared for publication as described at the beginning of the relevant chapter. Chapter 4 is written verbatim from the papers it is made up of. The author declares no conflict of interest in this thesis or the data it contains.

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Widjaja M[†], **Harvey KL[†]**, Hagemann L[†], Berry IJ, Jarocki VM, Raymond BBA, Tacchi JL, Gründel A, Steele JR, Padula MP, Charles IG, Dumke R*, & Djordjevic SP*. (2017) Elongation factor Tu is a multifunctional and processed moonlighting protein. Sci Rep 7: doi:10.1038/s41598-017-10644-z

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List of conference proceedings

Poster presentation 2016
17th International Symposium on Staphylococci and Staphylococcal Infections.
Seoul, Korea.

Poster presentation 2016
21st Lorne proteomics symposium.
Lorne, Australia.

Poster presentation 2015
New Horizons conference.
Sydney, Australia.

Poster presentation 2015
BacPath 13- Molecular Analysis of Bacterial Pathogens.
Phillip Island, Australia.

Poster presentation 2014
Proteomics and beyond symposium.
Sydney, Australia.

Poster presentation 2014
19th Lorne proteomics symposium.
Lorne, Australia.

Oral presentation and poster presentation 2013
The 5th Congress of European Microbiologists (FEMS 2013).
Leipzig, Germany.

Poster presentation 2013
18th Lorne proteomics symposium.
Lorne, Australia.

Lightening talk and poster presentation 2012
Proteomics and beyond symposium.
Sydney, Australia.

List of Abbreviations

General

Methicillin resistant *Staphylococcus aureus*	MRSA
Hospital-acquired methicillin resistant *Staphylococcus aureus*	HA-MRSA
Community-acquired methicillin resistant *Staphylococcus aureus*	CA-MRSA
Vancomycin intermediate *Staphylococcus aureus*	VISA
Vancomycin resistant *Staphylococcus aureus*	VRSA
Hospital acquired infection	HAI
United States	US
Small colony variant	SCV
Wild type	WT
Heparin	HP
Heparan sulfate	HS
Microbial surface components recognising adhesive matrix molecules	MSCRAMMs
Secretable expanded repertoire adhesive molecules	SERAM
Glycosaminoglycan	GAG
Proteoglycan	PG
Deoxyribonucleic acid	DNA
Ribonucleic acid	RNA
Gene ontology	GO
Extracellular deoxyribonucleic acid	eDNA
Glyceraldehyde 3-phosphate dehydrogenase	GAPDH
Centers for Disease Control and Prevention	CDC
Open reading frame	ORF
Intensive care unit	ICU
Single nucleotide polymorphism	SNP
Post-translational modifications	PTMs
Horizontal gene transfer	HGT
Colony forming units	CFU
Extracellular matrix	ECM
Whole cell lysate	WCL
Excreted cytosolic proteins	ECP
Elongation factor thermo unstable	Ef-Tu
Major histocompatibility complex class II analog protein	MAP
Short linear motif	SLiM
Protein-protein interaction	PPI

Method-related

Immuno-fluorescence microscopy	IFM
1-Dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis	1D-SDS-PAGE
2-Dimensional polyacrylamide gel electrophoresis	2D-PAGE
Difference in-gel electrophoresis	DIGE
Liquid chromatography	LC

Reversed-phase chromatography	RPLC
High pressure liquid chromatography	HPLC
Isobaric peptide tags for relative and absolute quantitation	iTRAQ
Isotope-coded affinity tags	ICAT
Stable isotope labels with amino acids in cell culture	SILAC
Hydrophilic interaction liquid chromatography	HILIC
Mass spectrometry	MS
Tandem mass spectrometry	MS/MS
Imaging mass spectrometry	IMS
Matrix assisted laser desorption/ionisation	MALDI
Electrospray ionisation	ESI
Electrospray droplet impact	EDI
Automated direct infusion	ADI
Time-of-flight	TOF
Strong cation exchange	SCX
Polymerase chain reaction	PCR
Semi-quantitative real-time polymerase chain reaction	sqrtPCR
Intelligent data acquisition	IDA

Solutions

Dithiothreitol	DTT
Acetonitrile	ACN
Trifluoroacetic acid	TFA
Sodium dodecyl sulfate	SDS
Phosphate buffered saline	PBS
Tryptic soy broth	TSB

Units used

Molar	M
Litre	L
Gram	g
Kilogram	kg
Second	s
Hour	h
Millimetres squared	mm^2
Nano	n
Milli	m
Micro	µ
Hertz	Hz
Kilo Dalton	kDa
Dalton	Da
Relative centrifugal force	rcf

Degrees celsius	°C
Potential of hydrogen	pH
Weight per volume	w/v
Optical density	OD
Parts per million	ppm
Molecular weight	MW
Amino acid	aa

Abstract

Staphylococcus aureus is a Gram-positive pathogen which causes a wide range of afflictions including endocarditis, osteomyelitis, cellulitis, toxic shock syndrome, and necrotising pneumonia. *S. aureus* is a leading cause of hospital-acquired infections and has rapidly acquired resistance to multiple antimicrobials. As such, it was deemed a serious threat by the Centers for Disease Control and Prevention (CDC) in 2013 indicating urgent attention is required to control this pathogen [1]. Currently there are no efficacious vaccines available to treat infections caused by *S. aureus*. With resistance being noted against every class of antibiotic currently available, the development of an alternative therapeutic would not only relieve morbidities, mortalities, and the associated economic burden, but also reduce the selective pressures that drive antibiotic resistance.

To better understand how *S. aureus* interacts with the human host and presents antigens that interact with key host cell receptors, a better understanding of which proteins are displayed on the cell surface is required. This dissertation presents an analysis of the surface proteome of *S. aureus* and describes several potential novel adhesins. Enzymatic cell shaving and surface protein biotinylation were used to catalogue proteins on the cell surface and identify regions within molecules that are surface accessible. Our approaches included methods that maintained protein size context (SDS-PAGE), providing an insight into the extent of surface protein processing. We also characterised heparin-binding proteins in *S. aureus* and interrogated the data in light of our surface proteome studies. This approach enabled us to gain insight into novel binding characteristics used by surface-accessible proteins that could not be predicted using reverse vaccinology and other hypothesis-directed approaches commonly used to develop potential vaccine candidates. Ascertaining the repertoire of heparin-binding proteins was considered important as these proteins are bacterial virulence

factors that facilitate adherence, colonisation, and invasion of target host cells. By coupling these data with other proteomic and bioinformatics techniques, a number of proteins of interest were identified. This includes Elongation Factor Tu, which was found to be surface exposed and highly processed, a finding that has not been seen before in *S. aureus*.

The data presented in the following chapters contributes significantly to the rapidly evolving field of *S. aureus* proteomics. These data will aid in the development of future therapeutic strategies and highlights a number of proteins for further therapeutic investigation.