The characterisation of

Shewanella algae

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UTS

Certificate of Original Authorship

I 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 as part of the collaborative doctoral degree and/or 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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Signature of candidate:

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Table of Contents

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List of Publications

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Melvold, J. A., Chowdhury, P. R., Padula, M. P., Djordjevic, S. P. and Charles, I. C., *The development of a proteogenomic pipeline to characterise the type VI secretory system (T6SS) of Gram-negative bacteria*, in *ASM Syntrophy*. 2015, Australian Socirty for Microbiology. p. 3.

Melvold, J. A., Wyrsch, E. R., McKinnon, J., Row Chowdhury, P., Charles, I. G. and Djordjevic, S. P., *The identification of a novel qnrA allele, qnrA8, in environmental Shewanella algae*, Journal of Antimicrobial Chemotherapy, 72(10):pp 2949-2952.

Publications in Review

Melvold, J. A., Row Chowdhury, P., Charles, I. G. and Djordjevic, S. P., *The draft genome of Shewanella algae SA2*, under review at Gut Pathogens.

Conference Proceedings

BacPath 13: Molecular Analysis of Bacterial Pathogens, Victoria 2015
Poster presentation
'The development of a proteogenomic pipeline to identify bacterial virulence factors'

The Australian Society for Microbiology, Annual Scientific Meeting, Canberra 2015
Oral presentation
'*The development of a proteogenomic pipeline to characterise the type VI secretory system of Gram-negative bacteria'*

The Australian Society for Microbiology Benton Dickson Awards, Sydney 2015
Oral presentation
'The development of a proteogenomic pipeline to characterise the type VI secretory system of Gram-negative bacteria'

Launch of AusGEM, Sydney 2014
Poster presentation
'Uncovering potential virulence factors of the emerging human pathogen Shewanella algae'

"Proteomics and Beyond" Symposium, Sydney 2014
Poster presentation
'Uncovering potential virulence factors of the emerging human pathogen Shewanella algae'

The 19th Lorne Proteomics Symposium, Victoria 2014
Poster presentation
'Investigations into the type VI secretory system of an emerging bacterial pathogen, Shewanella algae, using a proteogenomic approach'

BacPath 12: Molecular Analysis of Bacterial Pathogens, Queensland 2013
Oral presentation
'Development of a proteogenomic approach to characterise the Type VI secretory system (T6SS) of Gram-negative bacteria'

The 5th Congress of European Microbiologists (FEMS) Conference, Germany 2013
Poster presentation
'The secreteomes of indigenous Vibrio cholerae from Sydney water reveal pathogenic characteristics similar to O1 serotype Vibrio cholerae'

The 18th Lorne Proteomics Symposium, Victoria (Poster) 2013
'The secretomes of indigenous Vibrio cholerae from Sydney water reveal numerous pathogenic characteristics'

The Australian Society for Microbiology, Annual Scientific Meeting 2012
Poster presentation
'Shewanella algae: A wolf in sheep's clothing'

List of Figures

List of Tables

Abbreviations

1D-SDS-PAGE	One dimensional - sodium dodecyl sulphate - polyacrylamide gel electrophoresis
2D-SDS-PAGE	Two dimensional - sodium dodecyl sulphate - polyacrylamide gel electrophoresis
A5	Andrew and Aaron's Awesome Assembly pipeline
BHI	Brain heat infusion
BLAST	Basic local alignment search tool
CDS	Calibrated dichotomous sensitivity
DNA	Deoxyribose nucleic acid
E. coli	*Escherichia coli*
emPAI	Exponentially modified protein abundance index
HCl	Hydrochloride
Hcp	Hemolysin co-regulated protein
HMM	Hidden Markov models
LB	Luria Bertani
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LGT	Lateral gene transfer
m/z	Mass-to-charge ratio
MS	Mass spectrometry
MSHA	Mannose sensitive haemagglutinin
NSAF	Normalized spectral abundance factor
OD	Optical density
ORF	Open reading frame
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI	Propidium iodide
RAST	Rapid annotation subsystems technology

rpm	Revolutions per minute
S. algae	*Shewanella algae*
S. oneidensis	*Shewanella oneidensis*
S. putrefaciens	*Shewanella putrefaciens*
S. woodyi	*Shewanella woodyi*
SA1	Sydney strain of *Shewanella algae* SA1
SA2	Sydney strain of *Shewanella algae* SA2
SDS	Sodium dodecyl sulphate
SVM	Support Vector Machine
T6SS	Type VI secretion system
TBP	Tributylphosphine
TCBS	Thiosulfate citrate bile salts agar
V. cholerae	*Vibrio cholerae*
VgrG	Valine-glycine-repeat protein G

Abstract

The genus *Shewanella* comprises an extremely diverse group of facultative anaerobes that are widely distributed in freshwater and marine environments, including intertidal and benthic zones, their sediments and oil field wastes throughout the world [1, 2]. They are Gram-negative bacilli that are 1 - 2 μm in length and 0.4 - 0.7 μm in width which are motile via a single polar flagellum, exhibit un-paralleled respiratory diversity, and have robust sensing and regulatory systems which allow them to survive environments with low temperatures (less than 4°C), high salt concentrations and an extensive range of barometric pressures [3, 4]. These features lend themselves to phenotypic and physiological differences within the genus, but also have elicited interest in their use in biotechnology, including for bioremediation and microbial fuel cells [5, 6].

There are 63 species that comprise the *Shewanella* genus [7], and a handful of these are known to cause disease in humans and animals. The main species associated with human infection is *Shewanella algae* (*S. algae*) [1, 8], which naturally resides in aquatic environments and has been isolated from marine and freshwater sediments, oil fields, animals, marine life (including fish, sea lions, echinoderms, birds and poultry), and from human clinical material as the causative organism of diseases such as otitis media, cellulitis, septicemia and increasingly gastroenteritis [9-15]. To date, there have been limited studies investigating the mechanisms of pathogenicity and antibiotic resistance of *S. algae*.

The work presented in this dissertation has sought to address a number of gaps in knowledge regarding the pathogenesis of the emerging human pathogen *S. algae* using a systems biology approach. *S. algae* has the ability to cause mono-microbial infections in humans, ranging from infections of the skin and soft tissues, to blood borne and enteric infections. This thesis presents the first genome sequences of *S. algae* isolated from Sydney, Australia, and the first proteomic investigations which, combined, identify the presence and expression of potential virulence in this emerging human pathogen.

This dissertation has linked the *S. algae* genotype to the phenotype, giving a more holistic understand of the bacterium which is crucial to understanding any roles it has in pathogenesis. We identified a range of genes encoding putative virulence factors in *S. algae*, including toxins, haemolysins, adhesins, secretion systems, proteases and genes required for biofilm formation and motility/chemotaxis. Furthermore, the investigation into the expression of these proteins, via the differential growth media in the proteome and secretome, have highlighted that many of the genes encoding for these virulence factors require specific conditions for their expression.