Interrogating the microbiome for improved understanding of Pacific oyster diseases

William L King

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Certificate of Original Authorship

I, William King, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Publications associated with this thesis

Chapter One

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Chapter Two

King, W.L., Jenkins, C., Go, J., Siboni, N., Seymour, J.R., and Labbate, M. (2019). Characterisation of the Pacific Oyster Microbiome During a Summer Mortality Event. *Microbial Ecology*. 2019;77(2):502-12. 10.1007/s00248-018-1226-9

Chapter Three

King, W.L., Siboni, N., Williams, N.L.R., Kahlke, T., Nguyen, K.V., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R., and Labbate, M. (2019). Variability in the composition of Pacific Oyster microbiomes across oyster families exhibiting different levels of susceptibility to OsHV-1 μvar disease. *Frontiers in Microbiology* 10, 473. 10.3389/fmicb.2019.00473

Supporting Publication One

Green, T.J., Siboni, N., **King, W.L.**, Labbate, M., Seymour, J.R., and Raftos, D. (2018). Simulated Marine Heat Wave Alters Abundance and Structure of *Vibrio* Populations Associated with the Pacific Oyster Resulting in a Mass Mortality Event. *Microbial Ecology*. 10.1007/s00248-018-1242-9

Conference presentations associated with this thesis

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William L King, Nachshon Siboni, Michael Dove, Wayne O'Connor, Cheryl Jenkins, Justin R Seymour, Maurizio Labbate. Presentation titled: Polymicrobial involvement in OsHV-1 outbreaks (and other diseases). Oysters Australia Research and Development Meeting

William L King, Cheryl Jenkins, Jeffrey Go, Nachshon Siboni, Justin R Seymour, Maurizio Labbate. Presentation titled: Elucidating links between the Pacific Oyster microbiome and summer mortality events. 4th FRDC Australasian Scientific Conference.

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Abbreviations

C. gigas	*Crassostrea gigas*
S. glomerata	*Saccostrea glomerata*
O. edulis	*Ostrea edulis*
C. virginica	*Crassostrea virginica*
ROS	Reactive Oxygen Species
OsHV-1	Ostreid herpes virus 1
OsHV-1 μvar	Ostreid herpes virus 1 microvariant
P. marinus	*Perkinsus marinus*
H. nelsoni	*Haplosporidium nelsoni*
M. sydneyi	*Marteilia sydneyi*
B. roughleyi	*Bonamia roughleyi*
M. refringens	*Marteilia refringens*
B. ostreae	*Bonamia ostreae*
M. mackini	*Mikrocytos mackini*
R. crassostreae	*Roseovarius crassostreae*
N. crassostreae	*Nocardia crassostreae*
MSX	Multinucleate Sphere Unknown X
ROD	Roseovarius Oyster Disease
QX	Queensland Unknown
POMS	Pacific Oyster Mortality Syndrome
V. tubiashii	*Vibrio tubiashii*
V. splendidus	*Vibrio splendidus*
V. alginolyticus	*Vibrio alginolyticus*
V. aestuarianus	*Vibrio aestuarianus*
V. lentus	*Vibrio lentus*
V. harveyi	*Vibrio harveyi*
V. coralliilyticus	*Vibrio coralliilyticus*

V. crassostreae	*Vibrio crassostreae*
V. angillarum	*Vibrio angillarum*
V. diabolicus	*Vibrio diabolicus*
V. mediterranei	*Vibrio mediterranei*
V. azureus	*Vibrio azureus*
V. brasiliensis	*Vibrio brasiliensis*
V. chagasii	*Vibrio chagasii*
V. fortis	*Vibrio fortis*
V. vulnificus	*Vibrio vulnificus*
V. campbellii	*Vibrio campbellii*
V. sinaloensis	*Vibrio sinaloensis*
V. cholerae	*Vibrio cholerae*
V. parahaemolyticus	*Vibrio parahaemolyticus*
V. rotiferanus	*Vibrio rotiferanus*
ASI	Australian Seafood Industries
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
OTU	Operational Taxonomic Unit
ZOTU	Zero-radius Operational Taxonomic Unit
NSW	New South Wales
DPI	Department of Primary Industries
SRA	Sequence Read Archive
QIIME	Quantitative Insights Into Microbial Ecology
nMDS	Non-metric multidimensional scaling analysis
ANOVA	Analysis of Variance
ANOSIM	Analysis of Similarities
PERMANOVA	Permutational multivariate analysis of variance

CCA	Canonical Correspondence Analysis
SIMPER	Analysis of similarity percentages
PCoA	Principal Coordinates Analysis
RDP	Ribosomal Database Project
GIT	Gastrointestinal Tract
EBV	Estimated Breeding Values
RG	Resistance Group
qPCR	Quantitative Polymerase Chain Reaction
CV	Coefficient of Variation
WGS	Whole Genome Sequencing
FISH	Fluorescence in situ hybridization
MINE	Maximal Information-based Nonparametric Exploration
CR	Clyde River
GR	Georges River
HR	Hawkesbury River
SH	Shoalhaven
PS	Port Stephens
WA	Wapengo
Mt	Mantle
Gl	Gill
Am	Adductor muscle
Dg	Digestive gland
NCBI	National Center for Biotechnology Information
hsp60	Heat shock protein 60
BLAST	Basic Local Alignment Search Tool
NaCl	Sodium Chloride
LB	Lysogeny Broth
nt	Nucleotide

dNTP	Deoxyribonucleotide triphosphate
μL	Microlitre
μM	Micromolar
km	Kilometre
L	Litre
bp	Base pair
mg	Milligrams
ng	Nanograms

Abstract

Oyster aquaculture represents a significant portion of both the Australian, and the global economy, with *Crassostrea gigas* (the Pacific oyster) representing the most heavily cultivated commercial species. However, infectious diseases have emerged as a major obstacle for the successful growth and sustainability of the oyster aquaculture industry. Oyster diseases are often complex, occurring as a result of disturbance in the synergistic relationship between the host, environment, and pathogen/s. Perturbations of environmental factors (e.g. temperature, salinity, nutrients, pH) can have direct influences on the oyster's immune system, and can allow for the proliferation and transmission of oyster pathogens. In particular, two major pathogens of *C. gigas*, ostreid herpesvirus 1 (OsHV-1) and *Vibrio* species, are both strongly driven by temperature. One such understudied factor that may influence oyster disease dynamics is the oyster microbiome. Studies in other model systems have shown the involvement of the microbiome in animal health, disease, and behavior. Because of this, it is likely the oyster microbiome also plays a role in oyster disease dynamics. The work presented in this thesis aimed to use a microbiome approach to provide further understanding of oyster diseases.

Thesis prelude – rationale, significance and aims

The Australian aquaculture industry is valued at $1.31 billion AUD, representing 97,000 tonnes of production (ABARES, 2017). Of this, the oyster aquaculture industry contributes $97 million AUD, and 11, 300 tonnes of production (ABARES, 2017), making it a valuable contributor to the Australian economy. However, a major hurdle to the continued growth and sustainability of the oyster industry are infectious diseases (Lafferty et al., 2015).

Of the commercially cultivated oyster species, *Crassostrea gigas* (the Pacific oyster) is the most heavily cultivated globally (FAO, 2016a). Despite this, commercial cultivation of *C. gigas* has been continually challenged with disease outbreaks facilitated by viral, bacterial and unknown aetiological agents (Lipovsky, 1972; Paillard et al., 2004; Jenkins et al., 2013; King et al., 2019a). Current efforts to mitigate the impact of *C. gigas* diseases are focused on breeding for disease resistance (Dégremont, 2011; Dégremont et al., 2016b). This usually involves exposing oysters to disease in the field and breeding the surviving oysters (Dégremont, 2011). While breeding for disease resistance has been successful in reducing the impact of these diseases, the mechanism/s behind this protection are poorly understood.

Due to the economic importance of *C. gigas* cultivation, studies have sought to examine the causative factors driving these oyster disease outbreaks, with shifts in the environment (perturbations) often implicated as 'triggers' for disease (Burge et al., 2006; Malham et al., 2009; Jenkins et al., 2013; Mortensen et al., 2016; Go et al., 2017). These oyster diseases are complex, often preceding from a disturbance in the synergistic relationship between the host, environment, and pathogen. For example, shifts in environmental

conditions (such as increasing temperature) can drive oyster pathogen transmission and abundance (Petton et al., 2013), while also acting as an immune suppressant to the oyster, as they near their thermal limits (Bougrier et al., 1995). One such host associated factor that may be contributing to oyster disease dynamics is the oyster microbiome.

In recent years, the oyster microbiome has drawn an increasing amount of attention to determine its role in oyster disease dynamics (Lokmer and Wegner, 2015; Petton et al., 2015; Green et al., 2019; King et al., 2019a; King et al., 2019b; King et al., 2019c). However, at the onset of this PhD project, most disease-focused microbiome studies had been culture dependent (Garnier et al., 2007; Petton et al., 2013; Wendling et al., 2014; Lemire et al., 2015; Petton et al., 2015), with only one study employing culture-independent sequencing techniques (Lokmer and Wegner, 2015). Because of this and because of considerable *C. gigas* disease outbreaks in Australia in previous years (Jenkins et al., 2013; Go et al., 2017), this PhD project set out to gain further insight into the oyster microbiome.

This thesis has set out to address five aims, with the overarching goal to provide an improved understanding of oyster diseases using a microbiome approach. Ultimately, the information provided in this thesis will set the framework for future studies by identifying potential probiotic targets in the oyster microbiome and providing new microbiome approaches to oyster diseases for future disease-focused observational studies.

The below aims correspond to chapters one to five accordingly:

Aim one: To provide a critical review of oyster diseases, with an emphasis on the environmental drivers and the potential role of the microbiome.

Aim two: To use a microbiome approach to investigate a *C. gigas* disease outbreak in Port Stephens.

Aim three: To elucidate how breeding *C. gigas* for disease resistance influences microbiome composition and to identify bacteria associated with disease resistance.

Aim four: To determine microbiome patterns across geographic locations and tissue-types and identify core taxa innately tied to the *C. gigas* microbiome.

Aim five: To develop an amplicon sequencing assay for improved taxonomic resolution of the *Vibrio* community and to apply it to a laboratory *C. gigas* disease event.