Characterisation of the Sydney Rock Oyster microbiota and its association with QX disease

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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.

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This research was supported by an Australian Government Research Training Program.

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Abstract

The Sydney rock oyster (SRO; *Saccostrea glomerata*) is native to Australia and is the most intensively farmed oyster species in the country. However, diseases such as Queensland unknown (QX) disease have caused substantial losses and impeded productivity. The aetiological agent of QX disease is the parasite *Marteilia sydneyi* causing disease seasonally, generally in the late summer and early autumn. QX infection initiates at the palps and gills before migrating into the digestive gland where sporulation occurs causing blockage and eventual starvation and death in the oyster. Emerging evidence suggests that QX disease outbreaks are driven by a series of complex environmental and host factors such as salinity and oyster genetics. To mitigate the impacts of QX disease, the New South Wales Department of Primary Industries (NSW DPI) has led a selective breeding program using both mass selection methods and family-based breeding to create lines that are resistant. Some families have shown approximately 85% survival through one cycle of disease however, the mechanisms that drive this resistance are poorly understood. One potential factor influencing QX disease resistance is the microbiota which is investigated in this thesis.

In other oyster species, the microbiota is emerging as a key factor in disease dynamics and studies have begun to unravel the environmental factors that influence its structure such as location, season, genetics and disease state. For the SRO, there is a paucity of studies examining the microbiota and the factors that influence the SRO microbiota composition. Therefore, this thesis aimed to explore the influence of geographic location, season (comparing summer and winter) and genetics (selectively bred SROs with a range of resistance to QX disease) on the SRO microbiota (Chapter 2). It also investigated microbiota dynamics prior to and during a QX disease event (Chapter 3) and, compared the SRO microbiota across families for

identifying taxa that could be used as indicators for healthy and QX-infected oysters (Chapter 4).

Overall, this thesis provides new knowledge of the SRO microbiota and the factors that influence the SRO microbiota assemblage and contributes valuable information on how the SRO microbiota shifts during a QX disease event and identifies putative key taxa that may be important for health and disease states and/or good indicator taxa of these states. A better understanding of the SRO microbiota, its potential involvement in health and disease may enable effective disease prevention in the future.

Abbreviations

SRO: Sydney rock oyster

- C. gigas: Crassostrea gigas (also colloquially called the Pacific oyster)
- S. glomerata: Saccostrea glomerata (also colloquially called the Sydney rock oyster)
- O. edulis: Ostrea edulis (also colloquially called the European flat oyster)
- C. virginica: Crassostrea virginica (also colloquially called the Eastern oyster)
- OsHV-1: Ostreid herpes virus 1
- OsHV-1 µvar: Ostreid herpes virus 1 microvariant
- QX: Queensland Unknown
- WM: Winter mortality
- DPI: Department of Primary Industries
- NCBI: National Center for Biotechnology Information
- NSW: New South Wales
- SRA: Sequence Read Archive
- QIIME: Quantitative Insights Into Microbial Ecology
- nMDS: Non-metric multidimensional scaling analysis
- PERMANOVA: Permutational multivariate analysis of variance
- SIMPER: Analysis of similarity percentages
- STAMP: Statistical Analysis of Metagenomic Profiles
- SRO: Sydney rock oyster
- PCR: Polymerase chain reaction
- qPCR: Quantitative Polymerase Chain Reaction
- ISH: *in situ* hybridization
- PO: Phenoloxidase

OTU: Operational taxonomic unit

DNA: Deoxyribonucleic acid

RNA: Ribonucleic acid

rRNA: Ribosomal ribonucleic acid

dNTP: Deoxyribonucleotide triphosphate

μL: Microlitre

µM: Micromolar

km: Kilometre

L: Litre

bp: Base pair

mg: Milligrams

ng: Nanograms