The Pathology and Bacterial Ecology of Subtropical White Syndrome: A Disease of Scleractinian Corals in Subtropical Eastern Australia

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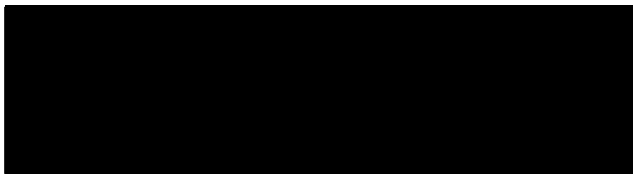
Declaration of Originality

I declare that the substance of this thesis is my own work. I certify that this thesis has not been submitted as part of another degree and is not currently being submitted for any other degree. Any help in preparing this thesis has been acknowledged and all sources have been cited.



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Abstract

Subtropical white syndrome (SWS) is an ecologically significant disease of scleractinian corals in shallow water benthic communities in subtropical eastern Australia. It causes mortality in a number of coral species and has the potential to seriously alter coral dominated communities in the region. This thesis describes the first investigation of the pathology and bacterial ecology of SWS in the model coral species *Turbinaria mesenterina*.

SWS typically presents as a progressive loss of tissue which originates from one point and gradually spreads across the coral colony. As the tissue is destroyed the white calcium carbonate skeleton underneath is exposed, resulting in a characteristic white lesion on the coral surface. Aquarium experiments using healthy and SWS affected *T. mesenterina* showed that SWS is an infectious disease that can be transmitted between colonies by direct contact, but is not transmitted via the water column. This finding implies that a vector must be involved in the transmission of the infectious agent from colony to colony. Further aquarium experiments showed that progression of SWS lesions is inhibited by antibiotics, indicating that a bacterial pathogen (or pathogens) is most likely responsible for the disease.

In order to better understand the role of bacteria in the SWS disease process, the bacterial communities associated with healthy and SWS affected *T. mesenterina* colonies were assessed using both culture-based and culture-independent methods. The work presented in this thesis is the first example the application of the Oligonucleotide fingerprinting of ribosomal genes (ORFG) method to a coral disease study. This method allows very large libraries of cloned bacterial 16S ribosomal genes to be constructed and analysed in a macroarray format without the need to isolate bacteria in culture. In this case, arrays of 9600 bacterial 16S genes were constructed that included representatives of the bacterial communities associated with both healthy and SWS affected *T. mesenterina*. The OFRG experiments showed that there were significant changes in the coral associated bacterial community as the coral moves through apparently healthy, diseased and dead states. In addition, a bacterial ribotype closely related to the known pathogen *Roseovarius crassostreae* was identified in association with diseased coral tissues.

The culture based assessment of the bacterial communities associated with healthy and SWS affected *T. mesenterina* also showed a clear succession of bacterial species as the disease progressed. Bacteria closely related to *Vibrio harveyi* were found in association with SWS lesions and are proposed as potential aetiological agents of the disease. Furthermore, both the culture-based and OFRG studies showed differences between the structure of the bacterial communities associated with the apparently healthy tissue in surviving areas of SWS affected colonies, and the tissue of completely healthy colonies unaffected by SWS, suggesting that infection with SWS involves changes in the microbial community of the entire coral colony.

Differences were also observed between the culturable bacterial communities associated with healthy *T. mesenterina* colonies from a near shore environment with extensive anthropogenic influences where SWS is present (Solitary Islands Marine Park) and an isolated offshore environment where SWS was not observed (Lord Howe Island). This observation provides support for the idea that shifts in the

structure of coral-associated bacterial communities helps corals adapt to changing environmental conditions.

The findings are discussed in the context of the coral holobiont concept, and a tentative model of the SWS infection process is proposed which incorporates all of the currently available information on SWS as well as evidence in the literature. In this model, it is proposed that an abiotic stressor such as elevated temperature causes shifts in the native bacterial community associated with healthy corals. This shift may reduce the coral's resistance to infection by lowering the numbers of beneficial bacteria which play defensive roles in the holobiont. The immunocompromised coral could then be infected by a pathogenic bacterium, carried by an animal vector, resulting in the development of SWS lesions. This model is critically discussed and avenues for future research are proposed to test this model and further the understanding of the pathology of SWS.

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List of Abbreviations

ºC	degree(s) Celsius
AGRF	Australian Genome Research Facility
Amp	ampicillin
Amp^R	ampicillin Resistant
BBD	Black Band Disease
BLAST	Basic Local Alignment and Search Tool
bp	base pair
BSA	Bovine Serum Albumin
CFU	Colony Forming Units
cm	centimetre
CSIRO	Commonwealth Scientific and Research Organisation
dATP	deoxyadenosine triphosphate
DF	Degrees of Freedom
DNA	Deoxyribonucleic Acid
EAC	East Australian Current
EDTA	ethylenediaminetetra-acetic acid
FSW	Filtered SeaWater
GCPAT	Greedy Clique Partition pAckage Tool
GBR	Great Barrier Reef
i.e.	that is
IPTG	isopropyl-β-D-1-thiogalactopyranoside
kb	kilobase (1000 base pairs)
km	kilometre
L	litre
LHI	Lord Howe Island
LB	Luria-Bertani medium
M	Moles per litre
M	metre
MEGA	Molecular Evolutionary Genetics Analysis
mg	milligram
min	minute(s)
µg	microgram
mL	millilitre
µL	microlitre
mM	millimoles per litre
µM	micromoles per litre
MYT	Marine Yeast-Tryptone medium
NCBI	National Center for Biotechnology Infomation
ng	nanogram
NMSC	National Marine Science Centre
NSW	New South Wales
NSWMPA	New South Wales Marine Park Authority
OFRG	Oligonucleotide Fingerprinting of Ribosomal Genes
OTU	Operational Taxonomic Unit
PAUP	Phylogenetic Analysis Using Parsimony
PCR	Polymerase Chain Reaction
rDNA/RNA	Ribosomal DNA/RNA
RDP	Ribosomal Database Project

RNA	Ribonucleic Acid
rpm	revolutions per minute
s	second(s)
SIMP	Solitary Islands Marine Park
SE	Standard Error
SLB	Salt Luria Broth
SONS	Shared OTU's and Similarity
SSI	Split Solitary Island
SWS	Subtropical White Syndrome
SWSI	Southwest Solitary Island
Tet	tetracycline
Tet^R	tetracycline Resistant
TRFLP	Terminal Restriction Fragment Polymorphism
U	Units of enzyme activity
UCR	University of California, Riverside
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UNE	University of New England
UV	Ultra Violet
VBNC	Viable But Not Culturable
v/v	volume to volume
WBD	White Band Disease
WP	White Plague
w/v	weight to volume
X-gal	5-bromo-4-chloro-3-indoyl β-D-galactopyranoside
YBD	Yellow Blotch/Band Disease

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