

Algal-bacterial interactions:
a study of *Gymnodinium catenatum*
and its associated bacteria

By

Maria Elisabeth Albinsson

M.Sc. Biology

Submitted in fulfilment of the requirement for the degree of
DOCTOR OF PHILOSOPHY

UNIVERSITY OF TASMANIA

Hobart, Australia

March, 2011

Declarations

This is to certify that this thesis contains no material that has been accepted for the award of any other degree or diploma in any tertiary institution. To the best of my knowledge this thesis does not contain any material published or written by another person, except when due reference is made in the text.

This thesis may be made available for loan. Copying of any part of this thesis is prohibited for two years from the date this statement was signed; after that time limited copying is permitted in accordance with the *Copyright Act of 1968*.



Maria Elisabeth Albinsson

1st March, 2011

Abstract

Over the last decades, harmful algal blooms (HABs) have increased globally in both frequency and extent, leading to intensified efforts to determine the primary factors that are controlling the population and toxin dynamics of bloom-forming algal species. One poorly understood factor in HAB ecology, yet shown to be of great importance, is that of algal-bacterial interactions.

Efforts to investigate the relationships and mechanisms of interaction are often hindered by the complex composition of the algal-associated bacterial communities, wherefore this present study used simplified algal-bacterial experimental model systems. The model systems are based on the toxin producing dinoflagellate *Gymnodinium catenatum* and two of its associated bacteria, *Marinobacter* sp. DG879 and *Alcanivorax* sp. DG881, both known to support growth and survival of *G. catenatum* in culture. Using the experimental model systems, the work presented in this thesis examined the growth dynamics of *G. catenatum* and its associated bacteria, and studied the effect of bacteria on the toxicity of *G. catenatum*. The abundance of *Alcanivorax* and *Marinobacter* genotypes associated with natural populations of *G. catenatum* in the Derwent Estuary and D'Entrecasteaux Channel, south-east Tasmania was also investigated.

This thesis demonstrates that the bacterial community is important for growth of *G. catenatum* and showed that different bacteria, in particular *Marinobacter* sp. DG879, have a significant effect on the growth dynamics and toxicity of *G. catenatum* laboratory cultures. When grown with *Marinobacter* sp. DG879, *G. catenatum* was found to remain viable in culture for a period longer than ever recorded, suggesting that *Marinobacter* sp. DG879 produce a compound inducing a positive growth response in *G. catenatum*, or that it plays a role in carbon recycling or nitrogen fixation. Toxin analysis of the *G. catenatum* / *Marinobacter* sp. DG879 cultures revealed a toxin profile significantly different to other analysed treatments containing different bacteria. As the experimental design of the study sought to minimise genetic variation among treatments, and with biotransformation generally operating on extracellular toxins (this study focussed on intracellular toxins), the differing toxin profile seen in

the *G. catenatum* with *Marinobacter* sp. DG879 is most likely a response to *Marinobacter* sp. DG879 influencing toxin synthesis indirectly through its effect on dinoflagellate physiology. The field studies also suggest that *Marinobacter* sp. may have an important role in natural populations of *G. catenatum*, preceding *G. catenatum* blooms.

With the use of molecular detection methods becoming more widespread, and with more research on naturally occurring blooms, there is potential that specific elements of the bacterial population, such as *Marinobacter* sp. DG879 could be employed as indicator species of *G. catenatum* and other algal blooms, ultimately allowing for early detection and management of potentially harmful blooms.

Acknowledgements

First and foremost I must thank my supervisors Dr. Christopher Bolch and Dr. Susan Blackburn.

Chris: Thank you for taking me on and putting up with me over these years, for giving me professional guidance, for sharing your knowledge and for showing me that steep learning curves are there to be dealt with.

Sue: Thank you for all your encouragement, support, time and afternoon chats, for being excited about my work, and for being there both as a supervisor, colleague and friend.

Thanks to the Aquafin CRC for financial support of my research, to the Thomas Crawford Memorial Scholarship allowing me to move half-way around the globe to do my PhD, and to the CSIRO Marine and Atmospheric Laboratories who have hosted me during these years.

I wish to thank Dr Andrew Negri (Australian Institute of Marine Science) for his help with toxin analysis and feedback on the final chapter.

A big thank you to Dr David Green (Scottish Association of Marine Science) for sharing his knowledge on algal bacterial interactions in experimental model systems

To everyone who helped me figure out PCRs and genetics: Stan Robert, Giles Campbell, Dion Frampton, Sharon Appleyard, James Wynne, Natasha Botwright, and Bronwyn Holmes, Thank you all!

I am very grateful to Cathy Johnston and Ian Jameson who have taught me and helped me in the CSIRO algal lab for many years now.

A big thank you to Alison Turnbull (Tasmanian Shellfish Quality Assurance Program/ Analytical Services Tasmania) for keeping me up-to-date on *G. catenatum* blooms.

A big thank you to all the guys who helped me with field samples, Dom O'Brien at Huon Aquaculture, Steve Percival at TASSAL and Grant Devine from Port Esperance Oyster farm.

I would also like to thank David Ratkowsky for his help with statistics, and Steven Myers for DNA sequences.

To all the people who have kept me sane: Giles, Jenn, Benita, Anna, Ben, Natasha, Peta and James, couldn't have done it without you guys!

Finally a big thank you to my family, Lena, Kenneth and Martin, for believing I could do this and for always supporting me no matter what I set out to do...

Table of contents

Declarations.....	ii
Abstract.....	iii
Acknowledgements.....	v
Table of contents.....	vi
List of figures.....	x
List of tables.....	xv
Chapter 1: Introduction.....	2
1.1 Introduction.....	2
1.2 Algal-Bacterial interactions.....	3
1.2.1 The Phycosphere.....	3
1.2.2 Algal-Bacterial associations and interactions.....	4
1.2.3 Experimental model systems of algal and bacterial interactions.....	6
1.3 <i>Gymnodinium catenatum</i> biology and ecology.....	8
1.3.1 Species description.....	8
1.3.2 Life cycle of <i>Gymnodinium catenatum</i>	10
1.3.3 Habitat, location and distribution.....	12
1.3.4 Toxicology of <i>Gymnodinium catenatum</i>	13
1.3.5 <i>Gymnodinium catenatum</i> -associated bacteria.....	16
1.4 Thesis aims and Outline.....	18
1.5 References.....	19
Chapter 2: Establishment of uni-bacterial <i>Gymnodinium catenatum</i> model systems: reproductive compatibility of <i>G. catenatum</i> strains, resting cyst production and germination.....	25
2.1 Introduction.....	25
2.2 Materials and Methods.....	27
2.2.1 Algal strains.....	27
2.2.2 Cyst production.....	28
2.2.3 Measurement of reproductive success.....	29
2.2.4 Bacterial strain maintenance and preparation.....	30

2.2.5	Surface sterilization of cysts and cyst germination.....	30
2.2.6	Establishment of <i>Gymnodinium catenatum</i> -bacterial model systems.....	31
2.2.7	Statistical analysis	31
2.3	Results	32
2.3.1	Reproductive compatibility of <i>Gymnodinium catenatum</i>	32
2.3.2	<i>Gymnodinium catenatum</i> resting cyst germination success.....	34
2.3.3	Establishment of <i>Gymnodinium catenatum</i> -bacterial model systems.....	35
2.4	Discussion	36
2.4.1	Reproductive compatibility of <i>Gymnodinium catenatum</i>	36
2.4.2	<i>Gymnodinium catenatum</i> resting cyst germination success.....	40
2.4.3	Establishment of <i>Gymnodinium catenatum</i> -bacterial model systems.....	41
2.5	References	43

Chapter 3: Development of and validation of a quantitative Real-time PCR assay for *Marinobacter* sp. and *Alcanivorax* sp. associated with *Gymnodinium catenatum*..... 46

3.1	Introduction	46
3.2	Materials and Methods.....	48
3.2.1	Bacterial cultures.....	48
3.2.2	DNA extraction	49
3.2.3	Design of TaqMan probe and primer sets.....	50
3.2.4	Specificity of TaqMan [®] probes and primers.....	56
3.2.5	Real time PCR assay conditions	59
3.2.6	Construction of standard curves.....	59
3.2.7	Detection limits	60
3.2.8	Algal cultures	60
3.3	Results	60
3.3.1	Specificity of TaqMan [®] probes and primers.....	60
3.3.2	Standard curves	70
3.3.3	Detection limits	73
3.4	Discussion	73
3.4.1	Comparison of TaqMan vs. SYBR Green.....	73
3.4.2	qPCR sensitivity.....	73
3.4.3	qPCR specificity	74
3.4.4	qPCR validation of quantification.....	76
3.5	References	77

Chapter 4: Use of real-time qPCR to track bacterial growth in uni-bacterial cultures of *Gymnodinium catenatum* 82

4.1	Introduction	82
4.2	Materials and methods	84
4.2.1	Algal and bacterial cultures.....	84
4.2.2	Calculations and statistical analysis	85
4.2.3	Real time PCR quantification of bacteria.....	86
4.3	Results	87
4.3.1	Growth dynamics	87
4.3.2	Bacterial proportions	91
4.3.3	Cell concentration correlations	93
4.3.4	Growth rates	99
4.4	Discussion	102
4.4.1	Growth dynamics of <i>Marinobacter</i> sp. DG879	102
4.4.2	Growth dynamics of <i>Alcanivorax</i> sp. DG881	103
4.4.3	Growth dynamics of <i>G. catenatum</i>	105
4.4.4	Nature of bacterial interactions with <i>G. catenatum</i>	107
4.4.5	Algal and bacterial cell concentration correlations.....	108
4.4.6	Growth rates of <i>G. catenatum</i> and bacteria.....	109
4.5	References	112

Chapter 5: Use of real-time PCR assays for detection of *Gymnodinium catenatum* growth-promoting bacteria in field samples 118

5.1	Introduction	118
5.2	Materials and methods	119
5.2.1	Field sampling	119
5.2.2	Sample preparation and filtration and cell counts.....	121
5.2.3	DNA extraction, Real time PCR assay conditions and sequencing	121
5.2.4	Statistical analysis	122
5.3	Results	122
5.3.1	Samples from CSIRO Wharf Station	122
5.3.2	Bloom samples	124
5.4	Discussion	127
5.4.1	Samples from Hobart CSIRO Wharf Station	128
5.4.2	<i>Gymnodinium catenatum</i> bloom samples	129
5.5	References	132

Chapter 6:	The effect of bacteria on the toxin production of <i>Gymnodinium</i>	
<i>catenatum</i>	136
6.1	Introduction	136
6.2	Materials and Methods	138
6.2.1	Algal and bacterial cultures	138
6.2.2	Production of resting cysts and unibacterial cultures	138
6.2.3	Sample preparation for toxin analysis	140
6.2.4	Calculations and statistics	141
6.2.5	HPLC analysis	142
6.3	Results	143
6.3.1	Growth of cultures	143
6.3.2	Toxin analysis	144
6.4	Discussion	153
6.4.1	Total amount of toxins in <i>G. catenatum</i>	153
6.4.2	Toxin production rate	154
6.4.3	Toxin profile	155
6.5	References	160
Chapter 7:	Summary and conclusion	165
7.1	Reproductive compatibility and the requirement for bacteria	166
7.2	Real-time PCR assays for bacterial enumeration	167
7.3	Bacterial influence on growth dynamics	168
7.4	Growth-promoting bacteria in field samples	170
7.5	The effect of bacteria on toxin production	171
7.6	Conclusions and aspects for future research	172
7.7	References	174
<i>Appendix 1: Preparation of GSe culture medium for dinoflagellates</i>		177
<i>Appendix 2: Preparation of ZoBell marine agar for bacteria</i>		180
<i>Appendix 3: <i>Gymnodinium catenatum</i> and bacterial strain list</i>		181