

Latitudinal study of the role of dimethylsulphoniopropionate in marine microbial foodwebs

By

Eva Fernandez Fernandez

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

School of life sciences
University of Technology Sydney
2020

“Caminante no hay camino. Se hace camino al andar”

“Traveller, there are no paths. Paths are made by walking”

Antonio Machado // Spanish poet
& Australian aboriginal saying

Certificate of authorship

I, Eva Fernandez Fernandez declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference 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.

This research is supported by the Australian Government Research Training Program

Signature:

Production Note:

Signature removed prior to publication.

Date: 15/06/2020

Acknowledgements

The time to wrap up my PhD adventure in Australia has arrived and so, it's time to thank all those people who made it possible. To all the new people who has come into my life the last 3.5 years and those who never left regardless the distance: Thank You for being there.

All my gratitude to my supervisor Dr. Katherina Petrou, who has always made me feel very welcome and comfortable and, has involved me in the best fieldtrips I could have ever imagined. Thanks to everybody who has been part of the MML team, even if briefly, you have always created a friendly, positive and collaborative atmosphere. Especial thanks to Belén, Alyson and Michelle, it has been a real pleasure to work with all of you.

Many thanks to some people of the C3 team for their help in the lab and valuable advice: James, Nashon, JB and specially my co-supervisor Justin Seymour for providing me with the opportunity to pursue my PhD in the interesting area of marine microbiology.

Thanks to everybody who have shared the path of pursuing a PhD with me: Nav, Caro, Ipek, Nasim and specially John for sharing our desk area.

My life in Sydney would have not been half as enjoyable as it has been without the friends I made in my new home country. Juan, my first year in Australia was the best year I could have. I really enjoyed exploring the outdoors with you, enjoy wherever you are. Samantha, it was good to find a bestie on the other side of the world to share a bottle of red with! So good that I have you! Also, many thanks to the snorkellers for great swims, laughs and good times. I also want to thanks my flatmate Michele not only for creating such a good working environment at home but for literally creating a home for both of us in our little Balinese house in Ersko.

And last but not least I want to thank my friends back home Barbara, Marina, Iria, Lili, UB Quimica (you know who you are) and my family for keeping in touch and made me feel like I had the best of both parts of the world.

Collection permits

Collection of Antarctic surface sea waters was permitted by the Australian Antarctic Division
(Permit IP16000336)

Table of Contents

CERTIFICATE OF AUTHORSHIP	III
ACKNOWLEDGEMENTS	IV
TABLE OF CONTENTS	VI
LIST OF FIGURES	X
LIST OF TABLES	XIII
ABSTRACT.....	17
CHAPTER 1: INTRODUCTION	20
1.1. Dimethylsulphonio propionate (DMSP) plays a crucial role in marine food webs, atmospheric fluxes and regional weather.....	20
1.1.1. The CLAW hypothesis	20
1.1.2. The sulfur cycle.....	21
1.1.3. The relevance of DMSP in marine food webs.....	23
1.1.4. Distribution of DMSP in world oceans	24
1.2. Dimethylsulfonylpropionate inside the cell: biosynthesis, uptake, degradation and function	256
1.2.1. Biosynthesis of DMSP	25
1.2.2. Suggested roles for DMSP inside the cell	28
1.2.3. DMSP uptake mechanism	300
1.2.4. Transformations of DMSP inside the heterotrophic cell: The bacterial switch	32
1.2.5. DMSP catabolic enzymes	33
1.3. Thesis objective and overview	35
CHAPTER 2: GENERAL METHODS	40
2.1. Analysis of sulfur compounds.....	40
2.2. Calculation of the relative abundances of DMSP degrading genes	434
2.3. Community diversity and composition	445
2.4. Analysis of ¹³ C-DMSP.....	467
2.5. Cell enumeration: Flow cytometry Analysis & Phytoplankton identification by light microscopy.....	50
2.6. Nutrients.....	501

2.7. Photophysiological condition of algae	501
2.8. Plankton biomass quantification	512
CHAPTER 3: DMSP UPTAKE AND ASSIMILATION BY NATURAL MICROBIAL COMMUNITIES OF THE GREAT BARRIER REEF (GBR), AUSTRALIA.....	
3.1 Abstract	534
3.2 Introduction	534
3.3. Experimental procedure and data analysis.....	578
3.3.1 Experiment 1: Quantification of rapid DMSP uptake by different fractions of the microbial community	589
3.3.2. Experiment 2: Effect of DMSP on community structure and gene regulation (long-term study)	60
3.3.3. Statistical analysis	612
3.4. Results	623
3.4.1 Characteristics of initial water masses.....	625
3.4.2 Experiment 1: Quantification of DMSP uptake by different fractions of the microbial community.....	645
3.4.2.1. Analysis of sulfur compounds: DMSPt, DMSPd, and DMSPp	645
3.4.2.2. Relative gene abundance and microbial composition.....	678
3.4.3. Experiment 2: Dynamic changes in microbial community composition and DMSP catabolising gene abundance	712
3.4.3.1. Analysis of sulfur compounds: DMSPt, DMSPd and DMSPp	712
3.4.3.2 Gene regulation/abundance	745
3.4.3.3. The influence of DMSP on 16S and 18S diversity	767
3.4.3.4. Correlation analysis and Principal Component Analysis	856
3.5. Discussion	878
3.5.1. Quantification of DMSP uptake by different fractions of the microbial community	889
3.5.2. Dynamic changes in microbial community composition and DMSP degradation genes abundance	934
3.6. Conclusions	978
CHAPTER 4: DISSOLVED DMSP UPTAKE AND ASSIMILATION BY MICROBIAL COMMUNITIES OF ANTARCTIC WATERS.....	
4.1. Abstract	1001
4.2. Introduction	1001
4.3. Experimental procedure.....	1034
4.3.1. Experiment 1: Quantification of rapid DMSP uptake by different fractions of the Antarctic microbial community	1034

4.3.2.	Experiment 2: Effect of DMSP on Antarctic community structure and gene regulation and the Identification of microorganisms that assimilate DMSP into their biomass.....	1045
4.3.3.	Statistical analysis.....	1067
4.4.	Results	1078
4.4.1.	Characteristics of initial water masses.....	1078
4.4.2.	Experiment 1: Quantification of DMSP uptake by different fractions of the microbial community.....	10910
4.4.3.	Experiment 2: Identification of microorganisms that assimilate DMSP into their biomass	1145
4.5.	Discussion	1245
4.5.1.	Quantification of DMSP uptake by different fractions of the microbial community	1245
4.5.2.	Dynamic changes in microbial community composition and DMSP degradation genes abundance	1278
4.6.	Conclusions	1301
 CHAPTER 5: REVEALING THE SULFUR CYCLERS ON PORT HACKING NATURAL SEA WATERS (NSW, AUSTRALIA).....		
		1334
5.1	Abstract.....	1334
5.2	Introduction	1334
5.3	Experimental procedure	1378
5.3.1.	Quantification of rapid DMSP uptake by the Port Hacking marine microbial community	1378
5.3.2.	Quantification of rapid DMSP uptake by different fractions of the Port Hacking marine microbial community	1389
5.3.3.	Statistical analysis.....	1389
5.4.	Results	13940
5.4.1.	Characteristics of initial water masses.....	13940
5.4.2.	Whole community incubation experiment	1401
5.4.3.	Fractioned community incubation experiment	1445
5.5.	Discussion	1501
5.5.1.	Quantification of DMSP uptake by the whole microbial community.....	1512
5.5.2.	Quantification of DMSP uptake by different fractions of the microbial community	1523
5.6.	Conclusions	1545
 CHAPTER 6: GENERAL CONCLUSIONS.....		
		1567
6.1	Uptake and distribution of DMSP in marine microbial communities of different latitudes ..	1567
6.2.	Effect of DMSP on the composition of marine microbial communities of different latitudes	15960

6.3. Future directions	1601
6.4. Concluding remarks.....	1612
REFERENCES.....	1634
APPENDIX A: SUPPORTING FIGURES AND TABLES FOR CHAPTER 1	185
APPENDIX B: SUPPORTING FIGURES AND TABLES FOR CHAPTER 2	1866
APPENDIX C: SUPPORTING FIGURES AND TABLES FOR CHAPTER 3	194
APPENDIX D: SUPPORTING FIGURES AND TABLES FOR CHAPTER 4	199

List of Figures

Figure 1. 1. The marine sulphur cycle.....	25
Figure 1. 2 DMSP distribution in the marine microbial food web.....	26
Figure 1. 3 Proposed DMSP biosynthetic pathways.....	28
Figure 1. 4 Schematic comparing the uptake and metabolism system for DMSP and acrylate in an α , β and γ proteobacteria.	31
Figure 1. 5 DMSP degradation pathways.	32
Figure 1. 6. Biochemical pathways for Dimethylsulphonio propionate (DMSP) degradation.	34
Figure 2.1 Schematic representation of the purge and trap system.....	41
Figure 2. 2 Water sampling scheme.	43
Figure 3.1. Location of sampling sites of initial water for experiments 1 and 2.	58
Figure 3.2. Experiment 1 flow chart.....	59
Figure 3.3. Experiment 2 flow chart.....	61
Figure 3.4. Nutrients concentrations of initial water for experiments 1 and 2.....	63
Figure 3. 5. DMSP concentrations and DMSP lyase activity over 7 h during experiment 1.....	67
Figure 3. 6. Relative abundance of DMSP degradation genes.....	68
Figure 3. 7. Bacterial and phytoplankton composition.....	69
Figure 3.8. Phylogenetic tree of eukaryotic (18S) community for the inner reef site.....	70
Figure 3. 9. Phylogenetic tree of eukaryotic (18S) community for the outer reef site.....	71
Figure 3. 10. DMSP concentrations and DMSP lyase activity during experiment 2.....	74
Figure 3. 11. Time course of the relative abundance of DMSP degrading genes from both experimental sites.....	76
Figure 3.12. Prokaryotic (16S) composition and nMDS plots for each experimental site..	79
Figure 3.13. Eukaryotic biodiversity, nMDS subset plot and phylogenetic trees for eukaryotic (18S) composition during experiment 2 for the outer reef site.....	81
Figure 3.14. Eukaryotic biodiversity, nMDS subset plot and phylogenetic trees for eukaryotic (18S) composition during experiment 2 for the outer reef site.....	83
Figure 3.15. Correlations of the relative abundance of DMSP-degrading genes and bacterial abundances for both sites.....	87

Figure 3.16. Principal Component Ordination (PCO) of bacteria composition for each site.....	88
Figure 4. 1. Location of sampling site of initial water masses for experiments 1 and 2.....	103
Figure 4.2. Experiment 1 flow chart.....	104
Figure 4.3. Experiment 2 flow chart.	106
Figure 4. 4. Nutrients concentrations of initial water masses for experiments 1 and 2.....	108
Figure 4.5. Example of common large (20 µm) Phytoplankton taxa identified in initial water samples for both experiments.....	109
Figure 4. 6. DMSP concentrations over 8 h during experiment 1.....	111
Figure 4.7. Abundance of DMSP degradation genes and microbial community composition.....	114
Figure 4. 8. Nutrient concentrations and maximum quantum yield of PSII (Fv/Fm) during experiment 2.....	115
Figure 4.9. DMSP concentrations during experiment 2.....	117
Figure 4.10. Abundance of DMSP degradation genes.....	118
Figure 4.11. Prokaryotic (16S) composition and nMDS plot during experiment 2.....	111
Figure 4.12. Eukaryotic biodiversity, nMDS subset plot and phylogenetic trees for eukaryotic (18S) composition during experiment 2.....	113
Figure 4.13. Correlation between DMSP total concentrations and Rhodobacterales relative abundance.	114
Figure 5.1. East Australian current (EAC) and oceanographic properties of Port Hacking station.	134
Figure 5.2. Location of sampling site and nutrients concentrations of initial experimental waters.....	137
Figure 5.3. Experiment flow chart.	191
Figure 5.4. Nutrients concentrations of initial experimental waters. Concentrations.....	139
Figure 5.5. DMSP concentrations of the whole marine microbial community over 17 h and nMDS plot.....	142
Figure 5. 6. Correlations between the different DMSP concentrations for control and +DMSP samples.....	143
Figure 5.7. Key Phytoplankton taxa identified in initial water samples.....	144

Figure 5.8. DMSP concentrations of fractionated marine microbial community over 6 h and nMDS plots.....	147
Figure 5.9. DMSPp at the final time point.....	149
Figure 5.10. Correlations between DMSP concentrations for each size fraction of the community.....	149
Figure 6.1. Major DMSP sinks for each climate.....	159

List of Tables

Table 1.1 DMSP total concentrations for different seas and times of the year.....	26
Table 1.2 DMSP concentrations in different organisms.....	27
Table.2.1. Operational settings of the GC-FPD for direct injection and purge and trap methods.....	41
Table 2.2. Primers and annealing Temperatures for the analysed genes.....	45
Table 3.1. Characteristics of initial water masses.....	64
Table 3.1. Major microbial groups and its contribution to treatment dissimilarity at the final time point (120h).	85
Table 4. 1 Characteristics of initial water masses.....	107
Table 4.2. Major microbial taxa and their contribution to treatment dissimilarity.....	123
Table 5.1. Sulphur chemistry and flow cytometric counts of microbial community.....	140
Table 5.2. Phytoplankton composition of the community.....	144
Table 5.3. Percentage and rate of DMSP loss/gain for each size fraction and treatment for DMSPt, DMSPd and DMSPp.....	148

Glossary of Terms

3HP	3-hydroxypropionate
ANOSIM	Analysis of Similarities
ANOVA	Analysis of Variance
ANSTO	Australian Nuclear Science and Technology Organisation
CE	capillary electrophoresis
CCN	cloud condensation nuclei
Chl a	Chlorophyll a
CLAW	Charlson-Lovelock-Andreae-Warren
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DLA	DMSP lyase activity
DMS	dimethyl sulphide
DMSHB	4-dimethylsulfonio-2-hydroxybutyrate
DMSO	Dimethyl sulfoxide
DMSP	dimethylsulfoniopropionate
DMSPd	dissolved DMSP
DMSPp	Particulate DMSP
DMSPt	Total DMSP
EA	Elemental analysis
F_v/F_m	potential quantum yield
FPD	flame photometric detector
GBR	The Great Barrier Reef
GBT	Glycine Betaine Transporter
GC	gas chromatography
GFF	glass fiber filter
GOS	Global Ocean Sampling
HPLC	High performance liquid chromatography
IAEA	Atomic Energy Agency (IAEA)

IMOS	Integrated Observing System
IR	Inside Reef
IRMS	Isotope ratio mass spectrometry
MC	Monte Carlo
MeSH	Methanethiol
MHM	MTHB methyltransferase
MMPA	methylmercaptopropionate
MS	mass spectrometry
MSNA	methane sulphinic acid
MTHB	4-methylthio-2-hydroxybutyrate
MTOB	4-methylthio-2-oxobutyrate
MTPA	3-methylthiopropylamine
MTA-CoA	methylthioacryloyl-CoA
nMDS	non-parametric multi-dimensional scaling
NMR	nuclear magnetic resonance
OR	Outside Reef
OTU	Operational taxonomic unit
PAM	pulse-amplitude-modulation
PCO	Principal Coordinates Analysis (PCO)
PCR	polymerase chain reaction
PERMANOVA	Permutational Multivariate Analysis of Variance
PH	Port Hacking
ROS	Reactive oxygen species
RV	Research vessel
SDS	sodium dodecyl sulfate
SMM	S-methyl-L-methionine
SO	Southern Ocean
SST	Sea surface temperature
TD	thermal desorption
UPLC	ultra-performance liquid chromatography

UV

Ultra violet

VPDB

Vienna Pee Dee Belemnite (VPDB)

Abstract

Dimethylsulphoniopropionate (DMSP) is a sulphur compound produced by some species of phytoplankton, coral and bacteria. It acts as a cryoprotectant, compatible osmolyte and antioxidant, and can provide high value nutrients for the whole marine microbial community. Nevertheless, research on DMSP has focused largely on its bacterial degradation to dimethylsulfide (DMS), a climatically active gas that potentially regulates local climate through an increase in cloud albedo. Therefore, other aspects of DMSP cycling, like DMSP utilisation by the marine microbial community, and especially by phytoplankton, are poorly understood. Marine sulphur dynamics, including DMSP production, cycling and DMS flux vary geographically across latitudinal space with different oceanographic characteristics resulting in different DMSP concentrations and microbial communities.

This thesis aimed to improve our understanding of the utilisation of DMSP by marine microbial communities from different oceanographic regions –the tropics (both coral-influenced and oligotrophic open waters), off shore temperate seas of mixed water masses, and late summer polar coastal waters— by investigating (1) the uptake of DMSP by different fractions of the marine microbial community, and (2) by identifying microbes that benefit from the presence of DMSP. This was achieved through a series of field-based studies that incubated natural oceanic waters enriched with DMSP over different time frames. Short incubations of 6-8 h were conducted to determine rapid DMSP uptake by the community following the progression of DMSP concentrations over time. The size separation of the community by serial filtration allowed for the quantification of DMSP that had been taken up by the microbes from the different size classes of the community. Longer incubations of up to 144 h were important for establishing longer-term responses, such as metabolism or fate of DMSP enrichment within the microbial community and DMSP-induced community shifts. Using sequencing information over time, we were also able to ascertain whether DMSP enrichment lead to any changes in DMSP metabolism and marine microbial structure.

Overall, the findings of this thesis challenge the idea that prokaryotes are the major DMSP sinks in the marine environment. Moreover, this thesis shows that phytoplankton uptake of DMSP is a common characteristic across different environments, with diatoms being one of the predominant sinks in the ocean. It has also shown that DMSP supposes an ecological

advantage to many bacteria and some phytoplankton taxa, highlighting the need for more research on DMSP degradation pathways in both bacteria and phytoplankton.