

ABSTRACT

Title of Dissertation:

**NOVEL BACTERIA ASSOCIATED WITH
TWO MICROALGAE OPTIMIZED FOR
CARBON DIOXIDE SEQUESTRATION
FROM FLUE GAS**

Lauren Elise Jonas, Doctor of Philosophy, 2025

Dissertation directed by:

Professor Russell T. Hill, Institute of Marine and
Environmental Technology, University of
Maryland Center for Environmental Science

The combustion of fossil fuels pollutes the atmosphere with carbon dioxide (CO₂) and contributes to global warming. Microalgae are significant players in the capture and transformation of CO₂ by way of their natural photosynthetic pathways. Moreover, the bacterial symbionts of microalgae can promote algal growth and resilience, thereby leading to additional carbon capture by microalgae. The significance of bacteria within microalgal systems is a significantly undervalued factor in microalgal research even though microalgae and bacteria are known to synergistically affect each other's growth and physiology. This dissertation focuses on the crucial and often unstudied contributions of bacteria in promoting microalgal growth. This work focuses on the role of bacteria in both small-scale (1 liter) and large-scale (500 liter, 6,800 liter) cultures of two microalgae, *Tetradesmus obliquus* strain HTB1 and *Nannochloropsis oceanica* strain IMET1 bubbled with simulated flue gas at 5% and 10% CO₂. Bacterial community analysis by using 16S rRNA gene sequencing of the

most productive algal cultures repeatedly revealed two dominant and novel bacteria with no taxonomic classification beyond the class level (Paceibacteria). Long read metagenomic sequencing yielded seven high quality metagenome assemble genomes (MAGs) of interest, including these two enigmatic unclassified bacteria. Two novel genera and species were taxonomically classified under the Seqcode (seqco.de/r:ywe1blo2): *Phycocordibacter aenigmaticus* gen. nov. sp. nov. and *Minusculum obligatum* gen. nov. sp. nov. The genus *Phycocordibacter* gen. nov. was proposed as the nomenclatural type of the family Phycocordibacteraceae fam. nov. and the order Phycocordibacterales ord. nov. In addition, functional analysis of plant growth promotion genes and metabolic reconstruction were conducted to elucidate how these bacterial symbionts promote microalgal growth and carbon fixation capacity. Epifluorescent microscopy and scanning electron microscopy (SEM) were conducted on both *N. oceanica* and *T. obliquus* cultures to understand the spatial relationship between each microalga and their respective bacterial symbionts. Digital PCR (dPCR) was conducted to quantify the ratio of microalgal cells to bacterial cells within cultures. To further improve CO₂ capture strategies using microalgae, understanding the taxonomy and functional impact of bacterial symbionts is vital.

**NOVEL BACTERIA ASSOCIATED WITH TWO MICROALGAE
OPTIMIZED FOR CARBON DIOXIDE SEQUESTRATION FROM FLUE
GAS**

by

Lauren Elise Jonas

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2025

Advisory Committee:

Professor Russell Hill, Chair
Associate Professor Yantao Li, Co-Chair
Professor Judith O'Neil
Professor Kevin Sowers
Professor Feng Chen

© Copyright by
Lauren Elise Jonas
2025

Dedication

To Anna, Isabel, Thomas, and Ethan

Acknowledgements

I would like to thank my family for all their love and support. Thank you, Mom, Dad, Louisa, Sam, Alex, Courtney, and Caroline for showing up for me. Thank you also to my nieces and nephews, Anna, Izzy, Tommy, and Ethan for being bright lights in my life.

The biggest thank you goes to my advisor, Dr. Russell Hill, who could not have been a more supportive advisor for both my master's and PhD. Thank you, Russell, for all the time and advice you have given me and for making me a better scientist and person. Your patience, kindness, and knowledge have made my time at IMET so valuable. I would also like to thank Dr. Yantao Li for agreeing to be my PhD co-advisor and for helping me at each step of my research and writing. Thank you to my committee members Dr. Judy O'Neil, Dr. Kevin Sowers, Dr. Feng Chen, and Dr. Zhongshi Liu for their guidance and insights into my research. Thank you to Dr. Tsvetan Bachvaroff for being such a wonderful person and for your guidance and chats. A big thank you to Sabeena Nazar for all of your hard work in the BAS Lab and for being so kind. Thank you to Kieran Szelong for being my Hill lab partner and friend. Thank you to Maurice White for all your help around IMET and for bringing positivity to the building. A big thank you to Jacqueline Joubert for your warmth, candy, and coffee hours! A big shoutout and thank you to Tessa Dumadag for taking such good care of my kittens which allowed me to take breaks from my research and travel.

My time at IMET would not have been the same without Drs(!) Daniela Tizabi, Ana Sosa, and Tori Camiener. Having you in and outside of IMET has been the greatest gift of graduate school and I love you three very much!

A big thank you to Dr. Diego Javier Jiménez Avella, who was my host and advisor at Universidad de los Andes in Bogotá Colombia. Thank you for your advice and push towards

metagenomic sequencing. This made all the difference in my project. I would also like to acknowledge the extraordinary help of both Dr. Tagide deCarvalho and Dr. Joseph Maubanm for their help in microscopy.

Outside of IMET, I am so lucky to have so many wonderful friends, all of whom fill up my life. Thank you, Anna Wherry, for making my time in Colombia (and Baltimore) so special and fun. Thank you to Erik Corbitt for being a good friend, cat sitter, and walking buddy. Caitlin Cromwell is the greatest friend I could ask for and I am grateful every day we are friends. Claire Murphy is my absolute rock and sister, thank you for everything. I would especially like to thank my partner, Cameron Clevidence, who is not only the most fun and calming person to be around, but also the best person I know.

Many names remain unmentioned here, but you all have made my time in graduate school incredibly rewarding. Thank you all.

Table of Contents

Dedication	ii
Table of Contents	v
List of Tables	ix
List of Figures	x
List of Abbreviations	xvi
Chapter 1: Introduction	1
1.1 Carbon sequestration and biotechnology using microalgae.....	1
1.1.1 Harnessing microalgae for sequestering carbon dioxide	1
1.1.2. Carbon fixation using microalgal biomass via high growth rates.....	2
1.2 <i>Tetradesmus obliquus</i> strain HTB1	4
1.2.1. <i>Tetradesmus obliquus</i> strain HTB1: nomenclature, physiology, and nutritional requirements.....	4
1.2.2 <i>T. obliquus</i> growth under various conditions.....	6
1.2.3 <i>T. obliquus</i> growth under elevated concentrations of CO ₂	8
1.2.4 Relevant omics work.....	10
1.3 <i>Nannochloropsis oceanica</i> strain IMET1	11
1.3.1 Review of research on growth of <i>Nannochloropsis</i> spp.	11
1.3.2 Genomics works on <i>Nannochloropsis</i> spp.....	13
1.3.3 <i>Nannochloropsis oceanica</i> strain IMET1	13
1.4 Bacterial communities associated with microalgae: benefits and considerations	15
1.4.1 The dynamics of symbiosis between microalgae and bacteria	15
1.4.2 Bacterial symbionts of HTB1 and IMET1	18
1.4.3 Sequencing bacterial communities and uncovering microbial dark matter	19
1.5 Aims of this dissertation	22
Chapter 2: Spatial associations between microalgae and their bacterial symbionts.....	23
2.1 Abstract	24
2.2 Introduction.....	26
2.2.1 Endosymbiotic associations of microalgae and bacteria	26
2.2.2 Bacterial symbionts residing in the microalgal “phycosphere”	27
2.2.3 Free-living bacterial symbionts of microalgae	28
2.2.4 Overview of this chapter	28
2.3 Methods.....	30
2.3.1 Light microscopy, epifluorescent microscopy, and scanning electron microscopy (SEM).....	30
2.3.2 Digital PCR (dPCR).....	32
2.3.3 Algal measurements using optical density and cell counting	33
2.4 Results.....	34
2.4.1 Light microscopy shows microalgal size and morphology.....	34
2.4.2 Epifluorescent microscopy.....	37
2.4.3 Scanning electron microscopy	42
2.4.4 Establishing ratios of microalgal cells:bacterial cells using digital PCR (dPCR)	47
2.5 Discussion	50

2.5.1 A distinction can be made between closely associated bacteria and free-living symbionts in microalgal cultures	50
2.5.2 Bacterial attachment and contributions to the phycosphere	51
2.5.3 Microscopy as a tool to visualize microalgal-bacterial consortia.....	52
2.5.4 Quantification of microalgae and bacteria.....	54
Chapter 3: Exploring the bacterial communities associated with microalgae through analysis of the 16S rRNA gene.....	56
3.1 Abstract.....	57
3.2 Introduction.....	58
3.2.1 Microalgal-bacterial consortia and mutualistic nutrient exchange	58
3.2.2 Antagonistic antics between microalgae and bacteria	60
3.2.3 Current research on industrially relevant microalgal-bacterial interactions	61
3.2.4 Overview of this chapter.....	63
3.3 Methods.....	65
3.3.1 Growth experiments and prokaryotic community analysis of <i>T. obliquus</i> HTB1	65
3.3.2 Growth experiments and prokaryotic community analysis of <i>N. oceanica</i> IMET1	66
3.3.3 Phylogenetic tree construction.....	67
3.4 Results.....	70
3.4.1 Bacterial communities associated with <i>Tetrademus obliquus</i> strain HTB1 at the 1 L scale.....	70
3.4.1 <i>Nannochloropsis oceanica</i> IMET1 bubbled with 10% CO ₂ or ambient air at the 1 L scale.....	74
3.4.2 Bacterial communities of <i>Nannochloropsis oceanica</i> IMET1 bubbled with air	75
3.4.3 Bacterial communities of <i>Nannochloropsis oceanica</i> IMET1 bubbled with 10% CO ₂	78
3.4.4 <i>N. oceanica</i> IMET1 and <i>T. obliquus</i> HTB1 bubbled with 5% CO ₂ at the 500 L and 6,800 L scale	81
3.4.5 Bacterial communities vary within different <i>N. oceanica</i> seed cultures	84
3.5 Discussion.....	88
3.5.1 Bacterial communities associated with <i>T. obliquus</i> at 1 L scale.....	88
3.5.2 Bacterial communities of <i>N. oceanica</i> at 1 L scale.....	89
3.5.3 Community composition changes within seed cultures, filtration methods, and scale up.....	90
3.5.4 Consistencies and dominance of two Patescibacteria.....	93
Chapter 4. Construction and analysis of metagenomic assembled genomes (MAGs) and nomenclature of two novel species, two novel genera, one novel family, and one novel order ..	94
4.1 Abstract.....	95
4.2 Introduction.....	96
4.2.1 Assembly of metagenome-assembled genomes (MAGs) from long-read sequencing data.....	96
4.2.2 Microbial dark matter and the nomenclature of uncultured prokaryotes.....	97
4.2.3 Validating taxonomic novelty and establishing nomenclature from sequence data	98
4.2.4 Overview of this chapter.....	100
4.3 Methods.....	101
4.3.1 Metagenomics library preparation and sequencing	101
4.3.2 Assembly of Metagenomic Assembled Genomes (MAGs).....	101

4.3.3 Phylogenetic analysis.....	104
4.3.4 Novelty determination and nomenclature.....	104
4.4.1 Details and statistics of sequencing rounds using the Oxford Nanopore MinION and GridION sequencer	106
4.4.2 Seven metagenome assembled genomes (MAGs).....	110
4.4.3 Establishing taxonomic novelty of two MAGs.....	132
4.4.4 Formal proposal of <i>Minusculum obligatum</i> gen. nov. sp. nov., <i>Phycocordibacter aenigmaticus</i> gen. nov. sp. nov., and associated higher taxonomic ranks.....	133
4.4.5 Description of <i>Minusculum obligatum</i> :	134
4.4.6 Description of <i>Phycocordibacter aenigmaticus</i> :	134
4.4.7 Description of Phycocordibacteraceae fam. nov.	135
4.4.8 Description of Phycocordibacterales ord. nov.	135
4.5 Discussion.....	142
4.5.1 Assembly of seven high quality MAGs from bacteria associated with two microalgal cultures.....	142
4.5.2 Unexpected and unprecedented dominance of novel Patescibacteria within microalgal cultures.....	144
4.5.3 Genome reduction and a lack of essential genes: familiar features of Patescibacteria	145
Chapter 5. Metabolic pathway reconstruction and functional gene analysis of bacteria associated with <i>Tetrademus obliquus</i> HTB1 and <i>Nannochloropsis oceanica</i> IMET1.....	146
5.1 Abstract.....	147
5.2 Introduction.....	149
5.2.1 Synergistic exchange between microalgae and bacteria.....	149
5.2.2 Harnessing the power of microalgal-bacterial consortia	151
5.2.3 Necessity for metagenomic sequencing and functional gene analysis to optimize various biotechnologies	152
5.2.4 Overview of this chapter.....	153
5.3 Methods.....	155
5.3.1 Metagenomics library preparation and sequencing	155
5.3.2 Metabolic pathway and lifestyle prediction.....	155
5.3.3 Annotation of amino acid biosynthesis pathways.....	155
5.3.4 Plant growth promotion gene analysis.....	156
5.4 Results.....	157
5.4.1 Metabolic prediction using protein-coding gene annotation with KEGG and HMM databases: <i>M. obligatum</i> and <i>P. aenigmaticus</i>	157
5.4.2 Metabolic prediction using protein-coding gene annotation with KEGG and HMM databases: <i>Gracilimonas sediminicola</i> , <i>Microcella pacifica</i> , <i>Algoriphagus aquaeductus</i> , <i>Allorhizobium</i> sp., and <i>Erythrobacter</i> sp.	159
5.4.3 Lifestyle predictions using symcla.....	165
5.4.4 Functional gene analysis using PGPT databases	166
5.5 Discussion.....	173
5.5.1 Nitrogen, sulfur, and iron metabolism within the <i>T. obliquus</i> and <i>N. oceanica</i> phycosphere	173
5.5.2 Amino acid auxotrophy prevalent in both <i>M. obligatum</i> , <i>P. aenigmaticus</i> , and the five well-characterized MAGs	176

5.5.3 Metabolic reconstruction reveals extreme streamlining within <i>M. obligatum</i> and <i>P. aenigmaticus</i>	178
5.5.4 Bacterial contribution to microalgal systems: Plant Growth Promotion Traits (PGPTs)	180
Chapter 6: Conclusions and Future Directions	183
6.1 The big picture: Improving the efficiency of biologically mediated carbon capture systems	183
6.2 Zooming in on both microalgal cultures: Striking and unexpected dominance of two novel Patescibacteria.....	185
6.3 Limitations and future directions	187
6.3.1 Limitations of working with uncultured Patescibacteria	187
6.3.3 Future Directions	189
6.4 Overall conclusions and final takeaways.....	192
Appendix to Chapter 2	195
Appendix to Chapter 3	197
Appendix to Chapter 4	204
Appendix to Chapter 5	207
Bibliography	214

List of Tables

Table 1.1 Summary of recent papers on the growth of <i>Tetradasmus (Scenedesmus) obliquus</i> using CO ₂ .	9
Table 2.1 Microscopes used to obtain micrographs of microalgae and their bacterial symbionts.	31
Table 2.2 Excitation and emission spectra in nanometers (nm) to detect autofluorescence in microalgae.	32
Table 3.1 Details on seven experiments chosen for bacterial community analysis	71
Table 3.2 Pairwise t-test of beta diversity between bacterial communities found within <i>T. obliquus</i> cultures.	73
Table 3.3 Sample details for Figure 3.12..	87
Table 4.1 Organizational map of tables and figures of this chapter.	106
Table 4.2 Sequencing statistics of six rounds of Nanopore sequencing.	108
Table 4.3 Statistics of successful sequencing rounds before and after filtering for high quality, determining minimum length cut-offs, and removing Nanopore sequencing adapters..	109
Table 4.4 Genome characteristics of the MAGs of this study.	131
Table 4.5 Comparison of the 16S rRNA gene sequence recovered from the same DNA extract sequenced with different platforms.	132
Table 4.6. List of proposed names and their current placeholders.	136
Table 4.7. Protologues of the MAGs of this study.	136
Table 5.1 Gene counts from major energy-producing pathways annotated with KEGG.	163
Table 5.2. Raw data output of the symcla lifestyle prediction model.	166
Table 5.3 Table displaying the NCBI's current full name of organisms abbreviated in heatmap in Figure 5.5.	171

List of Figures

Figure 1.1 <i>Tetrademus obliquus</i> HTB1 imaged with Keyence microscope.....	6
Figure 1.2 Growth of <i>Tetrademus obliquus</i> HTB1 cultures over 14 days.	9
Figure 1.3 Growth of <i>Nannochloropsis oceanica</i> IMET1 cultures over eight days	14
Figure 1.4 <i>Nannochloropsis oceanica</i> strain IMET1 imaged with Keyence microscope..	15
Figure 2.1 Micrographs of <i>Tetrademus obliquus</i> strain HTB1 taken by light microscopy at various dilutions and magnifications	35
Figure 2.2 Micrographs of <i>Nannochloropsis oceanica</i> strain IMET1 taken by light microscopy at various dilutions and magnifications.	36
Figure 2.3 Size distribution of <i>N. oceanica</i> IMET1 (blue) and <i>T. obliquus</i> HTB1 (red) using a Coulter Counter.....	37
Figure 2.4 Unstained <i>T. obliquus</i> HTB1 cells visualized with standard light microscopy (left) and excited by laser showing microalgal autofluorescence (right).....	38
Figure 2.5 <i>T. obliquus</i> HTB1 cells stained with DAPI.	39
Figure 2.6 <i>N. oceanica</i> IMET1 cells stained with DAPI visualized with standard light microscopy (left) and by laser (405 nm excitation/ 430-480 nm emission).	39
Figure 2.7 Epifluorescent micrographs of IMET1	40
Figure 2.8 Epifluorescent micrographs of HTB1.....	41
Figure 2.9 SEM micrographs of <i>N. oceanica</i> IMET1.....	43
Figure 2.10 SEM micrographs of <i>T. obliquus</i> HTB1.....	43
Figure 2.11 SEM micrographs of <i>N. oceanica</i> IMET1 and closely associated bacterial symbionts	44
Figure 2.12 SEM micrographs of <i>N. oceanica</i> IMET1 and free-living bacterial symbionts.	45
Figure 2.13 SEM micrographs of <i>T. obliquus</i> HTB1 and closely associated bacterial symbionts..	46
Figure 2.14 SEM micrographs of <i>T. obliquus</i> HTB1 and free-living bacterial symbionts.....	47

Figure 2.15 Digital PCR data displaying counts of <i>T. obliquus</i> HTB1 18S rRNA genes (green) and bacterial 16S rRNA genes (blue)	48
Figure 2.16 Digital PCR data displaying counts of <i>T. obliquus</i> HTB1 18S rRNA genes (green) and bacterial 16S rRNA genes (blue)	48
Figure 2.17 Digital PCR data displaying counts of <i>N. oceanica</i> IMET1 18S rRNA genes (green) and bacterial 16S rRNA genes (blue).	49
Figure 2.18 Digital PCR data displaying counts of <i>N. oceanica</i> IMET1 18S rRNA genes (green) and bacterial 16S rRNA genes (blue).	49
Figure 3.1 Conceptual diagram representing the microalgal phycosphere with various bacterial symbionts (left) and the nutrient exchange between both parties (right).....	60
Figure 3.2 Pipeline depicting filtering protocol, DNA extraction methods, and bioinformatic programs used for microbiome data analysis.....	69
Figure 3.3 Growth data of <i>Tetradismus obliquus</i> strain HTB1 grown with ambient air (black) and 10% CO ₂ (blue) at the 1 L scale.	72
Figure 3.4 Relative abundance (analysis of the V3/V4 region of the 16S rRNA gene) of closely associated (left 10 panels) and free-living (right 10 panels) bacteria in cultures of <i>Tetradismus obliquus</i> HTB1 grown with ambient air and 10% CO ₂	73
Figure 3.5 Performance of <i>Nannochloropsis oceanica</i> IMET1 cultures under ambient air (A-D, red lines) and 10% CO ₂ (E-H, green lines) in the presence or absence of 0.02 M NaHCO ₃ in 1 L column photobioreactors.....	75
Figure 3.6 Original published analysis showing relative abundance of closely-associated prokaryotic community (0.45 µm fraction – first ten bars) and free-living prokaryotic community (0.22 µm fraction – remaining ten bars) of <i>N. oceanica</i> IMET1..	76
Figure 3.7 Phylogenetic tree of partial 16S rRNA gene sequences associated with <i>N. oceanica</i> IMET1 microalgal cultures bubbled with ambient air.	77
Figure 3.8 Modified relative abundance plot based on Figure 3.6, with brown bars in Figure 3.6 now shown as two distinct bacteria: <i>Minuscolum obligatum</i> (dark teal) and <i>Phycocordibacter aenigmaticus</i> (light blue)..	78
Figure 3.9 Relative abundance plots of the closely associated prokaryotic community (0.45 µm fraction) and free-living prokaryotic community (0.22 µm fraction) of <i>N. oceanica</i> IMET1 through 16S rRNA gene sequence analysis.	80
Figure 3.10 Relative abundance plots of closely associated prokaryotic community (0.45 µm	

fraction) and free-living prokaryotic community (0.22 μm fraction) of *T. obliquus* HTB1 and *N. oceanica* IMET1 through analysis of the V3/V4 region of the 16S rRNA gene..... 83

Figure 3.11 Relative abundance plots of closely associated prokaryotic community (0.45 μm fraction) and free-living prokaryotic community (0.22 μm fraction) of *T. obliquus* HTB1 through analysis of the V3/V4 region of the 16S rRNA gene..... 84

Figure 3.12 Relative abundance plots of bacterial communities associated with various seed cultures of *N. oceanica* IMET1. y..... 86

Figure 4.1. Example output of the bioinformatic program, Nanoplot, from sequencing round, ‘MAG Round 3’. Average read quality (Phred quality score) vs. Read lengths (in base pairs, bp) of raw sequences. Sequences in red circle were removed from the analysis using NanoFilt due to both low quality and length (<Phred 10, <100 bp). 103

Figure 4.2 Circular genome view of *Phycocordibacter aenigmaticus*, a MAG recovered from cultures of *N. oceanica* IMET1 and *T. obliquus* HTB1.. 112

Figure 4.3 Phylogenetic inference of 16S rRNA genes taken from full genome sequences. *Phycocordibacter aenigmaticus* was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm..... 113

Figure 4.4 Circular genome view of *Minuscolum obligatum*, a MAG recovered from cultures of *N. oceanica* IMET1 and *T. obliquus* HTB1..... 115

Figure 4.5 Phylogenetic inference of 16S rRNA genes taken from full genome sequences. *Minuscolum obligatum* was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm..... 116

Figure 4.6 Phylogenetic tree deduced from alignment of partial 16S rRNA gene sequences (1,217 bp) of MAGs of this study (red) and nearest hits in the NCBI database. 117

Figure 4.7 Circular genome view of *Gracilimonas sediminicola*, a MAG recovered from cultures of *Nannochloropsis oceanica* IMET1..... 119

Figure 4.8 Phylogenetic tree deduced from alignment of partial 16S rRNA gene sequences (1,349 bp) of *Gracilimonas sediminicola* MAG of this study (blue) and nearest hits in the NCBI database..... 120

Figure 4.9 Circular genome view of *Microcella pacifica*, a MAG recovered from cultures of *Nannochloropsis oceanica* IMET1.. 122

Figure 4.10 Phylogenetic inference of 16S rRNA genes taken from full genome sequences. *Microcella pacifica* MAG of this study (blue) was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm.. 123

Figure 4.11 Circular genome view of <i>Algoriphagus aquaeductus</i> , a MAG recovered from cultures of <i>Tetradasmus obliquus</i> HTB1.	124
Figure 4.12 Phylogenetic inference of 16S rRNA genes taken from full genome sequences. <i>Algoriphagus aquaeductus</i> MAG of this study (blue) was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm....	125
Figure 4.13 Circular genome view of <i>Erythrobacter</i> sp., a MAG recovered from cultures of <i>Tetradasmus obliquus</i> HTB1.	127
Figure 4.14 Phylogenetic inference of 16S rRNA genes taken from full genome sequences <i>Erythrobacter</i> sp. MAG of this study (blue) was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm..	128
Figure 4.15 Circular genome view of <i>Allorhizobium</i> sp., a MAG recovered from cultures of <i>Tetradasmus obliquus</i> HTB1.	129
Figure 4.16 Phylogenetic inference of 16S rRNA genes taken from full genome sequences <i>Allorhizobium</i> sp. MAG of this study (blue) was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm..	130
Figure 4.17 Phylogenetic tree visualizing alignment of 120 concatenated universal bacterial genes generated by GTDB-Tk at the (A) phylum level, (B) class level, and (C) subsequent taxonomic ranks..	140
Figure 5.1 Heatmap [presence (green) absence (black)] displaying metabolic capacity of all seven MAGs.....	162
Figure 5.2 Heatmap displaying metabolic capacity of all seven MAGs predicted with METABOLIC-G.....	164
Figure 5.3. Symbiont scores of each MAG as determined by symcla.	165
Figure 5.4 Functional gene analysis from three rounds of metagenomic sequencing of <i>N. oceanica</i> cultures and one round of sequencing of <i>T. obliquus</i> cultures.	169
Figure 5.5 Heatmap displaying the correlation values of the most abundant species with the top functions within PGPT Level 3.....	170
Figure 5.6 Functional gene analysis from bacteria associated with <i>N. oceanica</i> IMET1 and <i>T. obliquus</i> HTB1.....	172
Figure A2.1 <i>T. obliquus</i> HTB1 cultures stained with DAPI and excited by laser (405 nm excitation/ 430-480 nm emission).....	195

Figure A2.2 <i>N. oceanica</i> IMET1 cultures stained with DAPI and excited by laser (405 nm excitation/ 430-480 nm emission).....	196
Figure A3.1 Alpha group significance using Faith's Phylogenetic comparing bacterial communities in <i>N. oceanica</i> samples receiving 10% CO ₂ and no NaHCO ₃ (0 M) and 0.02 M NaHCO ₃	197
Figure A3.2 Alpha group significance using Faith's Phylogenetic comparing bacterial communities in <i>N. oceanica</i> samples of different fraction sizes, 0.45 µm vs. 0.22 µm receiving 10% CO ₂	198
Figure A3.3 Alpha group significance using Faith's Phylogenetic comparing bacterial communities in <i>N. oceanica</i> samples receiving 10% CO ₂ across timepoints.....	199
Figure A3.4 Beta diversity analyzed with Unifrac distances (unweighted) comparing bacterial communities in <i>N. oceanica</i> samples receiving 10% CO ₂ across timepoints.....	200
Figure A3.5 Beta diversity analyzed with Unifrac distances (unweighted) comparing bacterial communities in <i>N. oceanica</i> samples receiving 10% CO ₂ of different fraction sizes, 0.45 µm vs. 0.22 µm.	201
Figure A3.6. Beta diversity analyzed with Unifrac distances (unweighted) comparing bacterial communities in <i>N. oceanica</i> samples receiving 10% CO ₂ receiving no NaHCO ₃ (0 M) and 0.02 M NaHCO ₃	201
Figure A3.7 Beta diversity analyzed with Unifrac distances (unweighted) comparing bacterial communities in 0.45 µm-fractionated <i>N. oceanica</i> samples receiving 10% CO ₂ supplemented with 0 M and 0.02 M NaHCO ₃ and 0.22 µm-fractionated samples supplemented with 0 M and 0.02 M NaHCO ₃	202
Figure A3.8. Photos of the 6,800 L bioreactor at HyTek Bio before collapse (left photo), containing <i>T. obliquus</i> (middle photo), and after collapse (right photo).	203
Figure A4.1. Output displaying pores available with the Nanopore chip sequencer during sequencing round, MAG4.	204
Figure A4.2. Assignment of protein features in <i>Minuscolum obligatum</i> (Patescibacterium UBA2103) by the Pathosystems Resource Integration Center (PATRIC), now provided through Bacterial and Viral Bioinformatics Resource Center (BV-BRC)..	204
Figure A4.3. Assignment of protein features in <i>Phycocordibacter aenigmaticus</i> by the Pathosystems Resource Integration Center (PATRIC), now provided through Bacterial and Viral Bioinformatics Resource Center (BV-BRC).	205
Figure A4.4 Output of assembly_info.fasta from Flye assembly algorithm (v 2.9.3) showing the major contigs of <i>Microcella pacifica</i>	206

Figure A5.1 Prediction of amino acid biosynthesis in <i>Minuscolum obligatum</i> using GapMind and the Uniprot identifiers.	207
Figure A5.2 Prediction of amino acid biosynthesis in <i>Phycocordibacter aenigmaticus</i> using GapMind and the Uniprot identifiers.	208
Figure A5.3 Prediction of amino acid biosynthesis in <i>Algoriphagus aquaeductus</i> using GapMind and the Uniprot identifiers.	209
Figure A5.4 Prediction of amino acid biosynthesis in <i>Allorhizobium</i> sp. using GapMind and the Uniprot identifiers.	210
Figure A5.5 Prediction of amino acid biosynthesis in <i>Erythrobacter</i> sp. using GapMind and the Uniprot identifiers.	211
Figure A5.6 Prediction of amino acid biosynthesis in <i>Gracilimonas sediminicola</i> using GapMind and the Uniprot identifiers.	212
Figure A5.7 Prediction of amino acid biosynthesis in <i>Microcella pacifica</i> using GapMind and the Uniprot identifiers.	213

List of Abbreviations

1,1,1,3,3,3-hexamethyldisilazane (HMDS)
4',6-diamidino-2-phenylindole (DAPI)
Ammonia (NH₃)
Amplicon sequence variants (ASVs)
Antimicrobial peptides (AMPs)
Average amino acid identity (AAI)
Average nucleotide identity (ANI)
Bacterial and Viral Bioinformatics Resource Center (BV-BRC)
Baltimore Back River Wastewater Treatment Plant (BRWWTP)
BioAnalytical Services Laboratory (BASLab)
Calcium carbonate (CaCO₃)
Carbon dioxide (CO₂)
Code of Nomenclature of Prokaryotes Described from Sequence Data (SeqCode)
Competitive Exclusion (CE)
Digital DNA–DNA hybridization (dDDH)
Digital polymerase chain reaction (dPCR)
Dimethyl sulfoxide (DMSO)
Dimethylsulfoniopropionate (DMSP)
Dissolved inorganic phosphate (DIP)
Dissolved organic carbon (DOC)
Dissolved organic carbon (DOC)
Elemental S (S⁰)
Enzyme Commission (EC)
Extracellular polymeric substances (EPS)
Federally Integrated Genomics Families (FIGfam)
Fluorescence in situ hybridization (FISH)
Gene Ontology (GO)
Genome BLAST Distance Phylogeny (GBDP).
Genome Taxonomy Database (GTDB)
Genomic relatedness indices (OGRIs)
Hidden Markov models (HMMs)
Hydrogen sulfide (H₂S)
Indole-3-acetic acid (IAA)
Institute of Marine and Environmental Technology (IMET)
Integrative Genome Viewer (IGV)

Interactive Tree of Life (iTOL)
International Code of Nomenclature of Prokaryotes (ICNP)
International Journal of Systematic and Evolutionary Microbiology (IJSEM)
Metagenome-Assembled Genome (MIMAG)
Metagenomic assembled genomes (MAGs)
Methicillin-resistant *Staphylococcus aureus* (MRSA)
Méthodes et algorithmes pour la bio informatique (MAB)
Microalga growth-promoting bacteria (MGPB)
Microbial Genomes Atlas (MiGA)
Minimum Information for Publication of Digital PCR Experiments (dMIQE)
Multigene-based phylogenies (MBPs)
N-acyl-homoserine lactone (AHL)
National Center for Biotechnology Information (NCBI)
Nitric oxide (NO)
Nitrite (NO₂⁻)
Nitrous oxide (N₂O)
Overall genome related indices (OGRIs)
Pathosystems Resource Integration Center (PATRIC)
PATRIC genus-specific family (PLfam)
PATRIC cross-genus family (PGfam)
Phosphate buffer solution (PBS)
Plant growth promotion (PGP)
Plant growth-promoting bacteria (PGPB)
Plant growth-promoting rhizobacteria (PGPR)
Plant growth-promotion traits (PGPTs)
PLant-associated BActeria web resource (PLaBAse)
Protein-coding sequences (CDS)
Quorum sensing (QS)
Relative evolutionary divergence (RED)
Scanning electron microscopy (SEM)
Short Read Eliminator (SRE)
Single-amplified genome sequences (SAGs)
Sodium bicarbonate (NaHCO₃)
Sulfite (SO₃²⁻)
Transmission electron microscopy (TEM)
Type Strain Genome Server (TYGS)

Chapter 1: Introduction

1.1 Carbon sequestration and biotechnology using microalgae

1.1.1 Harnessing microalgae for sequestering carbon dioxide

The impacts of climate change are intensifying, and the scientific community is urging for more microbial-based solutions (Peixoto *et al.*, 2024; Lennon *et al.*, 2025). Burning fossil fuels pollutes the atmosphere with carbon dioxide (CO₂) and contributes to climate change. In a review by Markewitz *et al.* (2012), the authors concluded that *ca.* 40% of worldwide CO₂-emissions originated from flue gas generated by fossil fuel power plants. This percentage has been cited frequently over time (Cuéllar-Franca and Azapagic, 2015; Singh *et al.*, 2019; Rao *et al.*, 2022; Yuan *et al.*, 2025), and is likely to have grown over the past decade. The cultivation of microalgae using power plant flue gas has become a useful method for carbon capture by way of natural photosynthetic pathways. Microalgae are promising candidates for carbon capture and utilization (CCU), a process in which CO₂ from flue gas is captured as microalgal biomass and can then be utilized for biofuel, fertilizer, feedstock, etc. (Kuckshinrichs, 2015). Microalgae are also used for carbon capture and storage (CCS) by which carbon is captured and stored as biomass or within an inorganic form, such as calcium carbonate (CaCO₃) (Cuéllar-Franca and Azapagic, 2015). We previously designed an algal system in which the CCU and CCS processes are combined in microalgae-driven calcium carbonate and biomass production (MadCAP), which relies on timed increases in culture pH and alkalinity leading to simultaneous production of algal biomass and CaCO₃ (Lee *et al.*, 2024). CCU, CCS, and the combined process (carbon capture utilization *and* storage, CCUS) using microalgae have become research hotspots due to

algae's high growth rate, tolerance to various environmental conditions, and the value of their molecular constituents as precursors to various products (Salih, 2011).

Algal molecular constituents—namely carbohydrates, proteins, and lipids—are valuable precursors to renewable crude oil, livestock feed, aquaculture feed, antioxidant supplements, agricultural fertilizers, and other industrial products (Daneshvar *et al.*, 2022). Further, unlike traditional crops used for biofuels (e.g. sugarcane, maize, rapeseed, etc.), microalgae do not take up valuable farmland and thus do not represent a threat to food production (Brennan and Owende, 2010). Microalgae can additionally be grown in non-potable water such as wastewater, brackish water, and seawater (Hoang *et al.*, 2022). For example, microalgae have been shown to efficiently treat various types of wastewater such as industrial, agricultural, and anaerobic digestion effluent (Ruiz-Marin *et al.*, 2010; Arbib *et al.*, 2012; Cai *et al.*, 2013; Ruiz *et al.*, 2014; Shen *et al.*, 2015; Ho *et al.*, 2019). Moreover, secondary-treated municipal wastewater has been used for the cultivation of several species of microalgae (Ruiz *et al.*, 2011; Álvarez-Díaz *et al.*, 2015; Gómez-Serrano *et al.*, 2015; Ho *et al.*, 2019). Studies on microalgal wastewater treatment have often been paired with algal biofuel development, and various species of microalgae have been identified as attractive candidates for generating biofuels due to their fast growth rate and high carbon capture efficiency (Liu *et al.*, 2017; Hoang *et al.*, 2022). The simultaneous benefits of (1) growing microalgae with wastewater, (2) supplying the cultures CO₂ from flue gas, and (3) producing biofuel and other value-added products make microalgae substantial players in environmental mitigation strategies.

1.1.2. Carbon fixation using microalgal biomass via high growth rates

The promising benefits of algal biotechnology are conditional on supplying algae with CO₂ for photosynthesis and carbon fixation. This can be provided in the form of smokestack

exhaust which would otherwise contribute to air pollution and global warming. In general, the percentage of CO₂ in flue gas from thermal power plants ranges from 3% to 15%, but is most commonly around 10% (Packer, 2009; Yadav *et al.*, 2019). Not all species of microalgae can withstand the high concentration of CO₂ in power plant flue gases; many microalgae grow only under atmospheric CO₂ concentrations of approx. 0.042%. Within the literature generally, concentrations of 2–5% CO₂ are described as “high”, 5–20% is considered as “very high”, and 20–100% is commonly referred to as “extremely high” (Miyachi *et al.*, 2003; Moghimifam *et al.*, 2020). Under elevated CO₂ conditions (any concentration above atmospheric conditions), photosynthetic/carbon fixation efficiency can decline due to electron transport capacity or the limited activation state of the RuBisCO enzyme (Sage *et al.*, 1988). High concentrations of CO₂ can also lower pH of the surrounding medium which can lead to growth inhibition, and this can be mitigated with the addition of alkaline solutions (Lee *et al.*, 2000, 2024; Jiang *et al.*, 2013).

The tolerance of microalgal species to elevated concentrations of CO₂ falls along a spectrum. Enhanced growth at 10% or higher CO₂ concentrations has been reported for a number of algal species, including *Chlamydomonas reinhardtii* (Yang and Gao, 2003; Choi *et al.*, 2021), *Chlorella* sp. (Yang and Gao, 2003; Doucha *et al.*, 2005; Westerhoff *et al.*, 2010; Kumar *et al.*, 2014; Duarte *et al.*, 2017), *Spirulina* sp. (Kumar *et al.*, 2010a; Duarte *et al.*, 2020), *Euglena gracilis* (Chae *et al.*, 2006), *Chlorogleopsis* sp. (Ono and Cuello, 2007), *Botryococcus braunii* (Ge *et al.*, 2011; Rodas-Zuluaga *et al.*, 2021), *Dunaliella* spp. (Moghimifam *et al.*, 2020), *Scenedesmus* sp. (Yang and Gao, 2003; Westerhoff *et al.*, 2010; Jiang *et al.*, 2013; Liu *et al.*, 2019; Rodas-Zuluaga *et al.*, 2021), and *Nannochloropsis* sp. (Negoro *et al.*, 1993; Jonas, Y. Lee, *et al.*, 2025). Other studies have demonstrated successful cultivation of dense microalgal biomass using simulated flue gas or direct effluent from fossil fuel powerplants (Negoro *et al.*, 1993;

Yoshihara *et al.*, 1996; Lee *et al.*, 2000; Kumar *et al.*, 2014; Cheah *et al.*, 2015; Aslam *et al.*, 2017; Duarte *et al.*, 2017).

Microalgal growth can be inhibited by continuous injection of flue gas due to the accumulation of heavy metals and NO_x and SO₂ compounds, although several studies have found that microalgae can effectively remove NO_x and SO₂ compounds with little harm to cell growth and lipid production (Lee *et al.*, 2002; Rahaman *et al.*, 2011; Yen *et al.*, 2015). The promise of two particular genera—*Tetradesmus* (formerly *Scenedesmus*) and *Nannochloropsis*—in CO₂ fixation, growth rate, and production of value-added products are discussed in the following sections. I used two species of microalgae in this study. *Tetradesmus obliquus* HTB1 was isolated by the laboratory of Dr. Feng Chen from the Back River, a tributary of the Chesapeake Bay (Liu *et al.*, 2013). *Nannochloropsis oceanica* IMET1 was obtained from Greece, maintained and grown on a large scale by the National Center of Mariculture, Eilat, Israel (Lubzens *et al.*, 1995) and distributed to many laboratories including the Institute of Marine and Environmental Technology (IMET) in Baltimore, MD (Chi and Takiguchi, 2015).

1.2 *Tetradesmus obliquus* strain HTB1

1.2.1. *Tetradesmus obliquus* strain HTB1: nomenclature, physiology, and nutritional requirements

One promising microalga for carbon capture and utilization is *Tetradesmus obliquus* (Figure 1.1). Formerly assigned the taxonomy *Scenedesmus obliquus* (Turpin) Kützing 1833 and *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata 2000, the currently accepted nomenclature is *Tetradesmus obliquus* (Turpin) M. J. Wynne 2016 (Wynne and Hallan, 2016).

For a full history of the taxonomic reclassifications, see Cardon *et al.*, 2018 or Oliveira *et al.*, 2021.

Tetradismus is a freshwater algal genus that is found across various aquatic environments. *T. obliquus* cells are ovoid, 4–5.8 µm in diameter, and are often found in a cluster of four cells (Smith, 1913). The cells contain one chloroplast as well as a single pyrenoid in the chloroplast (Cardon *et al.*, 2018). Pyrenoids are organelles that enhance photosynthesis by providing the key photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) with concentrated CO₂ (He *et al.*, 2023). Recent studies have demonstrated the potential large-scale biomass production of *T. obliquus* due to its fast growth rate under photoautotrophic, heterotrophic, and mixotrophic conditions (Olivera *et al.*, 2021 and references therein). High biomass of *T. obliquus* can be achieved by supplementing cultures with an organic carbon source that fulfils nutritional requirements, energy requirements, or both (Girard *et al.*, 2014; Vieira, 2018; Di Caprio *et al.*, 2019). It has been suggested that when cultivated through mixotrophic metabolism, *T. obliquus* may achieve its highest growth rate (Girard *et al.*, 2014; Shen *et al.*, 2018). However, no studies have tested this hypothesis with algae grown under identical light exposure, temperature, culture medium, and cultivation systems. Not only is photoautotrophic growth the most common method of cultivation for all commercial microalgae species, but it is also a cost effective method for large-scale production and has great potential for the mitigation of elevated concentrations of CO₂ in the atmosphere (Iglina *et al.*, 2022). Photoautotrophic growth, supplementing algal cultures with elevated concentrations of CO₂ to mimic the concentrations found in flue gas effluent, is the only growth method explored in this dissertation.

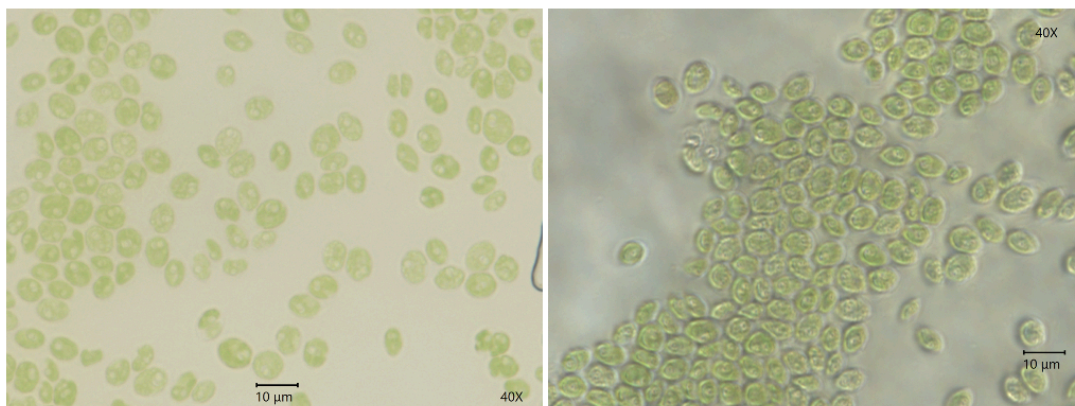


Figure 1.1 *Tetradesmus obliquus* HTB1 imaged with Keyence microscope. Methods are described in Chapter 2, Section 2.3.1.

1.2.2 *T. obliquus* growth under various conditions.

T. obliquus has shown great potential to grow in adverse conditions, such as in secondary treated wastewater (Martínez *et al.*, 1999), urban wastewater (Martínez *et al.*, 2000; Kong *et al.*, 2021), brewery effluent (Mata *et al.*, 2012), and olive oil production wastewater (Hodaifa *et al.*, 2008). The strain HTB1 specifically has achieved high growth (10.2 g L^{-1}) when supplemented with nutrient extracted from chicken manure (Han *et al.*, 2017). A detailed review of *T. obliquus* cultivation in various sources of wastewater can be found in Oliveira *et al.*, 2021. *T. obliquus* has also been shown to grow in brackish water, which is non-potable and unable to be used in most agricultural or industrial processes. However, the aim of those studies was to assess the potential for desalinization and increased lipid production under conditions of higher salinity (Gan *et al.*, 2016; Wei *et al.*, 2020). Algal growth rates consistently decreased as salinity increased (Gan *et al.*, 2016). Trace metal concentrations seemed to not be a key factor in *T. obliquus* growth for a study found that cultures grown with trace metal concentrations 10-, 100-, and 1000-times less than that of control media all reached *ca* 4.3 g L^{-1} on the tenth day of cultivation (Oliveira *et al.*, 2020). *T. obliquus* has also demonstrated unique tolerance to high temperatures and light. Most

microalgae, including *T. obliquus*, can grow at temperatures between 15 and 40°C. Highest growth rates of *T. obliquus* have been recorded at 25°C (Ma *et al.*, 2019; Zhang *et al.*, 2019) 27.5°C (Breuer *et al.*, 2013), 29.5°C (Hodaifa *et al.*, 2010), and 30°C (Martínez *et al.*, 1999; Guedes *et al.*, 2011). Even when temperature reached 40°C, one study showed that no growth inhibition of *T. obliquus* was recorded (Venancio *et al.*, 2020).

T. obliquus is tolerant to various light intensities and wavelengths of light, apart from green light which is inefficiently absorbed by green-pigmented chlorophyll (Hurtado *et al.*, 2019). In two separate studies in which bioreactors were illuminated with varying light intensities (ranging from 10 to 1,000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), *T. obliquus* reached its maximum growth at 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Gris *et al.*, 2014; Sforza *et al.*, 2014). Other studies have shown an ideal irradiance of 420 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Ho *et al.*, 2012), 434 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Hindersin *et al.*, 2014), and 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Ho *et al.*, 2014). When irradiance was increased from 360 to 1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 4.2 g/L biomass was achieved after 10 days (Oliveira *et al.*, 2020). The lack of consensus for *T. obliquus*'s ideal light conditions is most likely due to the differing cell densities that different studies use in the initial inoculum. Cultures with lower cell densities are more vulnerable to excessive illumination, a phenomenon known as the photoinhibition effect where both CO₂ fixation rate and biomass productivity can be inhibited by high irradiance (Han *et al.*, 2000). However, increased cell densities can cause cell self-shading, which can make light a limiting factor for growth in dense cultures. Increasing light intensity as microalgal cultures grow is key to minimize impacts of both photoinhibition early in growth and cell self-shading once dense cultures have developed (Hindersin *et al.*, 2013, 2014; Oliveira *et al.*, 2020). Additionally, constant illumination (as opposed to light/dark cycling) has been shown to produce the highest biomass in *T. obliquus* (4.02 g L⁻¹) (Vendruscolo *et al.*, 2019).

1.2.3 *T. obliquus* growth under elevated concentrations of CO₂

T. obliquus has shown great promise in CO₂ fixation, growth rate, and production of value-added products. Over the course of 14 days, *T. obliquus* HTB1 cultures grew to higher densities when bubbled with 10% CO₂ than with ambient air (Figure 1.2). When *T. obliquus* was grown under 0.038%, 6%, 12%, and 18% CO₂, its biomass (assessed by dry weight measurement) was the highest at 12% after 21 days (de Moraes and Costa, 2007c). The same group found that *T. obliquus* also grew well (1.12 g/L after 20 days) when the culture medium contained up to 18% CO₂ (de Moraes and Costa, 2007c). Tang et al. (2011) found that growth at 10% CO₂ concentration produced the highest biomass (1.84 g/L dry weight) in *T. obliquus* strain SJTU-3 after 14 days (Tang *et al.*, 2011). In another study of *Scenedesmus* sp., the biomass concentration (as well as carbohydrates and protein production) was at its highest (1.95 g/L dry weight) at 20% CO₂ after 15 days (Rodas-Zuluaga *et al.*, 2021). The highest biomass achieved for *T. obliquus* is with 10% CO₂ with biomass reaching 3.51 and 3.13 g/L after 12 and 14 days, respectively (Ho *et al.*, 2010; Yoo *et al.*, 2010). *T. obliquus* has been found not only to accumulate biomass at high CO₂ conditions, but when sparged with simulated flue gas [including 100 ppm nitric oxide (NO)], approx. 97% of NO and 88% of CO₂ was assimilated into algal biomass (Ma *et al.*, 2019). These results demonstrate that *Scenedesmus* sp., particularly strains of *T. obliquus*, are promising candidates for carbon sequestration and NO_x removal from smokestack flue gas. These findings and additional studies are summarized in Table 1.1.

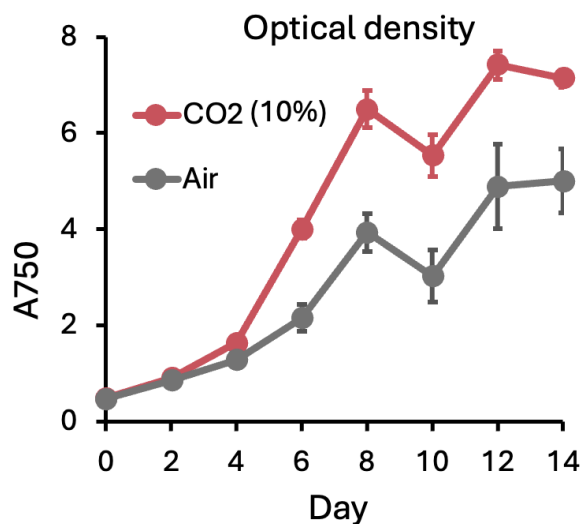


Figure 1.2 Growth of *Tetradesmus obliquus* HTB1 cultures over 14 days measured by optical density (absorbance at 750 nm). Culture set-up and methods are described in Chapter 2, Section 2.3.3.

Table 1.1 Summary of recent papers on the growth of *Tetradesmus* (*Scenedesmus*) *obliquus* using CO₂.

Algae (Name used in publication)	CO ₂ (%)	Biomass (g/L)	Productivity (mg L ⁻¹ day ⁻¹)	Specific growth rate “μ _{max} ” (g/day)	Publication
<i>Scenedesmus obliquus</i> strain CNW-N	10	3.51	292.50	n/a	(Ho <i>et al.</i> , 2010)
<i>Scenedesmus obliquus</i> strain LEB 22	0.038	ca .1.4	n/a	0.29	(De Morais and Costa, 2007b)
	6	ca. 1.8	160	0.33	

	12	2.12	n/a	0.33	
	18	ca. 1.5	n/a	0.28	
<i>Scenedesmus obliquus</i> LEB 22	12	1.14 ± 0.01	76 ± 0.001	0.249 ± 0.021	(De Morais and Costa, 2007c)
	18	1.12 ± 0.01	74 ± 0.001	0.260 ± 0.025	
<i>Scenedesmus obliquus</i> LEB 22	6	1.81	100	0.22	(de Morais and Costa, 2007a)
	12	1.80	140	0.22	
<i>Scenedesmus</i> sp. KCTC AG2083	10	3.13	217.5 ± 11.24	n/a	(Yoo <i>et al.</i> , 2010)
<i>Scenedesmus obliquus</i> SJTU-3	10	1.84	155 ± 0.004	0.887 ± 0.012	(Tang <i>et al.</i> , 2011)
<i>Scenedesmus</i> sp. (UTEX)	20	1.95	n/a	ca. 0.57	(Rodas-Zuluaga <i>et al.</i> , 2021)
<i>Scenedesmus obliquus</i> PF3	5	2.55 ± 0.14	260 ± 0.01	n/a	(Ma <i>et al.</i> , 2019)
	10	2.54 ± 0.13	270 ± 0.01		
<i>Scenedesmus obliquus</i>	10	0.76	n/a	0.23	(Liu <i>et al.</i> , 2020)
<i>Scenedesmus obliquus</i>	15	2.3	145	n/a	(Kaewkannetra <i>et al.</i> , 2012)

1.2.4 Relevant omics work

Three draft genomes for *Tetradasmus obliquus* have been registered with the National Center for Biotechnology Information (NCBI), one at the scaffold level and two at the contig

level.¹ Further, a complete mitochondrial DNA sequence has been published (Nedelcu *et al.*, 2000). One transcriptomic study has been conducted with HTB1 grown with air enriched with 10% CO₂. HTB1 was found to have an enhanced photosynthetic efficiency and biomass accumulation under 10% CO₂ conditions, without displaying any significant metabolic changes in carbon fixation and ATP synthesis (M. Zhang *et al.*, 2022).

1.3 *Nannochloropsis oceanica* strain IMET1

1.3.1 Review of research on growth of *Nannochloropsis* spp.

The genus *Nannochloropsis* is also of interest due to the ability of strains to grow at high CO₂ concentrations. *Nannochloropsis* is a genus of microalgae that is widely distributed in marine, brackish, and freshwater environments, and consists of at least six species: *N. oceanica*, *N. oculata*, *N. salina*, *N. limnetica*, *N. granulata* and *N. gaditana* (Andersen *et al.*, 1998; Li *et al.*, 2014; Ashour *et al.*, 2019). *Nannochloropsis* members are unicellular, range between 2–8 µm in diameter, and have a chloroplast in close proximity to the nucleus with stacks of thylakoids similar to plant cells (Ma *et al.*, 2016).

Species of *Nannochloropsis* have been studied extensively for aquaculture feed, nutritional supplements for livestock, and biofuel production (Cheng-Wu *et al.*, 2001; Jia *et al.*, 2015; Liu *et al.*, 2017; Esteves *et al.*, 2021). These algae are of interest due to their high content of storage lipids such as triacylglycerols (TAGs) and the omega-3 fatty acid, eicosapentaenoic acid (EPA), as well as carotenoids (Lubián *et al.*, 2000; Rodolfi *et al.*, 2009; Van Vooren *et al.*, 2012). Production of EPA tends to correlate with favorable growth conditions, while

¹ These genomes are also registered under previous names, *Scenedesmus obliquus* and *Acutodesmus obliquus*.

accumulation of TAG correlates with light stress and nutrient deprivation (Wang and Jia, 2020, and references therein). The majority of research has focused on enhancing growth and lipid production through manipulation of light, temperature, nutrient availability, photobioreactor types, etc. For example, Sandnes *et al.* (2005) found the growth rate of *N. oceanica* peaks within the temperature range of 25–29°C. The increased growth rate was directly proportional to increased irradiance over a range of 34–80 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, with the optimal conditions being 28°C at 80 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. In a study testing the effects of light stress, *N. oceanica* achieved its highest biomass (*ca.* 8 g/L) when illuminated with 200 $\mu\text{mol m}^{-2} \text{ s}^{-2}$, compared with 50 and 500 $\mu\text{mol m}^{-2} \text{ s}^{-2}$ (Wang and Jia, 2020).

Less research attention has been paid to CO₂ sequestration (particularly when compared to studies on *T. obliquus*), however many strains of *Nannochloropsis* show strong growth when bubbled with high concentrations of CO₂. For example, *N. oceanica* IMET1 cultures grew to higher densities when bubbled with 10% CO₂ than with ambient air (Figure 1.3, Jonas *et al.*, 2025a). *Nannochloropsis* sp. aerated with 15% CO₂ achieved a biomass of 1.43 g/L after 10 days, which was significantly higher than that of control cultures bubbled with ambient air (0.39 g/L) (Jiang *et al.*, 2011). In another study, 8% CO₂ was the ideal concentration for *N. oculata*, yielding 1.66 g/L after 18 days; the cultures also achieved high biomass (1.36 g/L) at 12% CO₂ concentration (Razzak *et al.*, 2015). Adamczyk *et al.* (2016) cultivated *N. gaditana* under 4% and 8% CO₂ for ten days and achieved biomass concentrations of 4.02 and 4.05 g/L, respectively. Further, *N. gaditana* was found to have a CO₂ fixation rate of more than 1.7 g/L/day under 8% CO₂ conditions. These values were significantly higher than biomass concentrations of *Chlorella* sp. grown under the same concentrations (Adamczyk *et al.*, 2016). When grown with simulated flue gas, *Nannochloropsis* sp. biomass was recorded at over 2 g/L (ash-free dry

weight) after six days of bubbling with 15% CO₂ supplemented with 100 and 300 ppm NO (Yoshihara *et al.*, 1996). One study suggests that optimal CO₂ concentrations for *N. oceanica* growth may depend on light intensity. *N. oceanica* achieved its highest biomass (2.45 g/L) when subjected to 80 $\mu\text{mol photons m}^{-2} \text{ s}^{-2}$ paired with 50% CO₂. However, when illuminated with 60 $\mu\text{mol photons m}^{-2} \text{ s}^{-2}$, the algae grew better with air enriched with 30% CO₂ (Rao *et al.*, 2022).

1.3.2 Genomics works on *Nannochloropsis* spp.

Within the NCBI database, seven genomes have been published for *Nannochloropsis* spp. (Radakovits *et al.*, 2012; Corteggiani Carpinelli *et al.*, 2014; Wang *et al.*, 2014) including *N. oceanica* (Pan *et al.*, 2011; Vieler *et al.*, 2012; Hu *et al.*, 2014).

1.3.3 *Nannochloropsis oceanica* strain IMET1

N. oceanica strain IMET1 was originally obtained from Eilat, Israel and has been maintained at the Institute of Marine and Environmental Technology (IMET) for more than 30 years (Figure 1.4). IMET1 has been identified as a promising strain for algal biofuel production because of its rapid lipid accumulation under nitrogen depletion conditions (Xiao *et al.*, 2013). Most research on IMET1 focuses on the enhancement of lipid production under different conditions of nitrogen stress and will not be reviewed in this dissertation. One study compared both IMET1 and *N. gaditana* strain CCMP526 in microplates supplemented with 0, 2, 10, 15, and 20% CO₂. While the growth rates of IMET1 and *N. gaditana* were the highest at a 2% CO₂, IMET1 outperformed *N. gaditana* under all conditions (Chi and Takiguchi, 2015). However, it should be noted that IMET1 growth was inhibited in this study when bubbled with 15 and 20% CO₂. Several genomic (Hu *et al.*, 2014; Wang *et al.*, 2014), proteomic (Dong *et al.*, 2013), metabolomic (Xiao *et al.*, 2013), transcriptomic (Li *et al.*, 2014; Wang *et al.*, 2014), and

lipidomic (Li *et al.*, 2014) studies have been performed on IMET1, providing an excellent foundation for future research.

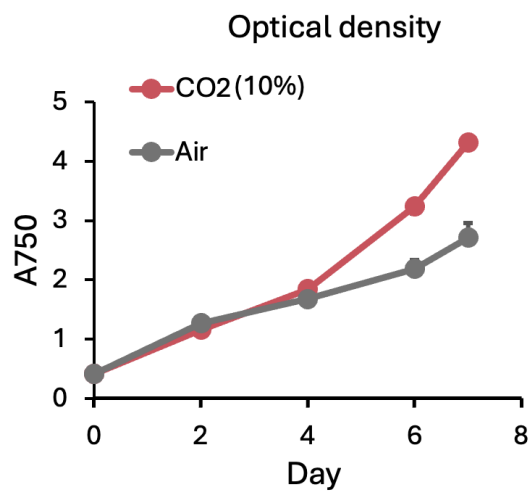


Figure 1.3 Growth of *Nannochloropsis oceanica* IMET1 cultures over eight days measured by optical density (absorbance of 750 nm). Culture set-up and methods are described in Chapter 2, Section 2.3.3.

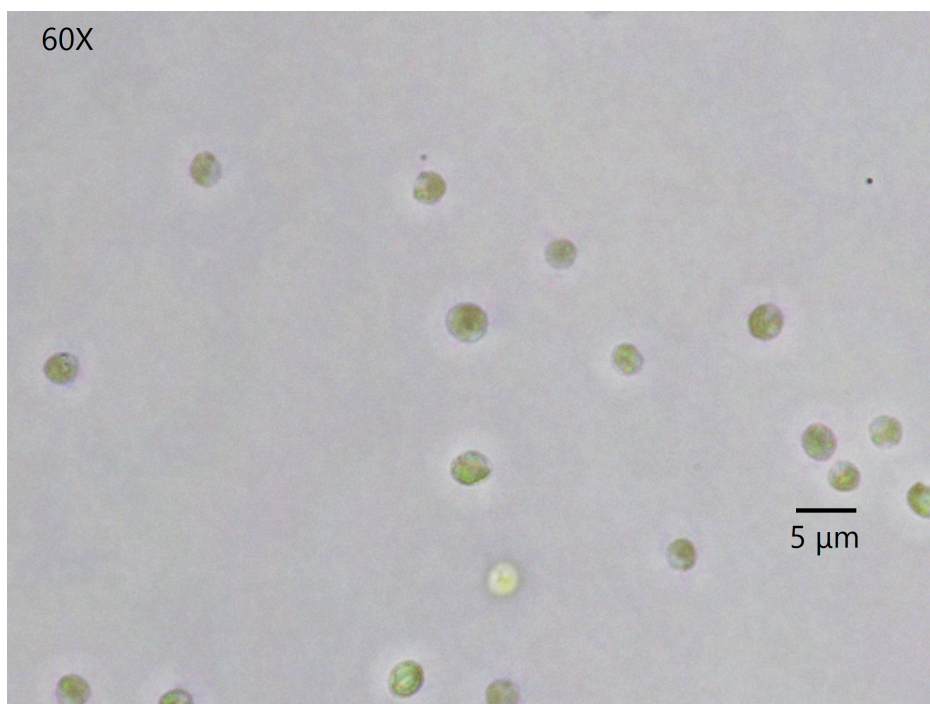


Figure 1.4 *Nannochloropsis oceanica* strain IMET1 imaged with Keyence microscope. Methods are described in Chapter 2, Section 2.3.1.

1.4 Bacterial communities associated with microalgae: benefits and considerations

1.4.1 The dynamics of symbiosis between microalgae and bacteria

Understanding and harnessing the symbiosis between microalgae and bacteria has become a focus within microalgal biotechnology because of the various benefits that bacteria can provide to microalgal cultures. The location of the bacterial cells in these symbiotic relationships occurs in four categories. Bacteria can (1) exist as free-living cells that live within a close range of algal cells or (2) live within an extracellular polymeric substance (EPS) matrix that is secreted by microalgae, termed the ‘phycosphere’ (Bell and Mitchell, 1972). Bacteria can also (3) be

externally attached to algal cells or bacterial cells can (4) be fully integrated into the host cell and subsist as endosymbionts. The reliance on the algal host seems to be closely related to the type of physical contact they have with their bacterial symbionts. While endosymbiotic relationships have been found to be obligatory, looser associations tend to be more facultative and can be temporary rather than long-lasting (Mayali *et al.*, 2011; Zachar and Boza, 2020). In this study, algal-bacterial associations have been classified into two categories: “closely-associated” symbionts, including attached symbionts and endosymbionts, are retained with the microalgae on filtration through a 0.45-micron filter. Subsequently, “free-living” symbionts pass through the 0.45-micron filter and are collected on a 0.22-micron filter.

Several mutually beneficial exchanges between microalgae and bacteria have been described in natural and laboratory systems. One well-studied example is nutrient exchange. In aquatic systems, heterotrophic bacteria can obtain a large fraction of their carbon demand directly from nearby algae and/or cyanobacteria. In fact, up to 50% of the fixed carbon can be ultimately consumed by bacteria (Fuhrman and Azam, 1982; Fouilland *et al.*, 2014). Algae can also release highly labile dissolved organic carbon (DOC) into the water that can then be consumed by nearby bacteria (Larsson and Hagström, 1979; Piontek *et al.*, 2011; Seymour *et al.*, 2017). Similarly, particulate organic matter (POM) can be released from algal cells through protist grazing and viral lysis (Biddanda and Pomeroy, 1988). Heterotrophic bacteria can benefit greatly from these sources of dissolved nutrients and large polysaccharides. In return, bacteria can provide algae with remineralized nutrients that are otherwise limiting (Seymour *et al.*, 2017). Another major contribution of bacterial symbionts to algae is vitamin B₁₂, which is required for the synthesis of methionine. More than 53% of the algal kingdom are B₁₂-auxotrophs (Croft *et al.*, 2005); they either use a B₁₂-independent form of methionine synthetase or obtain exogenous

B₁₂ that is produced by nearby bacteria. Croft et al. (2005) experimentally showed that *Halomonas* sp. was solely responsible for supplying vitamin B₁₂ to the algae *Amphidinium operculatum* and *Porphyridium purpureum*.

Many microalgal cultures are maintained in co-culture with bacteria due to the potential for bacteria to promote algal growth. These bacteria have been termed “Microalga Growth-Promoting Bacteria (MGPB)” and reviewed in depth (Palacios *et al.*, 2022). Some notable examples of MGPB include *Bacillus pumilus* that was shown to promote growth of *Chlorella vulgaris* by supplying fixed atmospheric nitrogen (Hernandez *et al.*, 2009). *Phaeodactylum tricornutum* cell density rose by 55% in the presence of an Alphaproteobacterium strain (Bruckner *et al.*, 2011). *Pelagibaca bermudensis* and *Stappia* sp. promoted the growth of marine microalga *Tetraselmis striata* (Park *et al.*, 2017). The growth of a green alga *Dunaliella* sp. increased by 22% and 26% when co-cocultured with *Alteromonas* sp. and *Muricauda* sp., respectively (Le Chevanton *et al.*, 2013). Certain growth-promoting bacteria produce plant growth hormones called auxins, and some of these bacteria (such as *Rhizobium*, *Azospirillum* sp., and *Sphingomonas*) have been widely identified in algal cultures (Kloepper, 1980; Ramanan *et al.*, 2015). *Azospirillum* sp., produces a specific auxin called indole-3-acetic acid (IAA) that is transferred to *Chlorella* sp. and *Scenedesmus obliquus* strain C1S, consequently promoting growth (Gonzalez and Bashan, 2000; De-Bashan *et al.*, 2008; Meza *et al.*, 2015; Pagnussat *et al.*, 2020). IAA has also been shown to enhance the growth of *Emiliania huxleyi* by 25%, although higher IAA concentrations can also cause algal lysis (Segev *et al.*, 2016). The synthesis of algal-growth promoting auxins by bacteria and its implications for the future for algal biotechnology has been reviewed by Lin *et al* (2022).

1.4.2 Bacterial symbionts of HTB1 and IMET1

A handful of studies have been conducted (several at IMET) that investigate the interactions of bacteria with this dissertation's two algal strains of interest, HTB1 and IMET1. Dr. Shailendra Singh of the Li Lab at IMET found that two bacterial strains, *Rudanella* sp. and *Cloacibacterium normanense* strain IMETF, were able to promote HTB1 growth (Singh *et al.*, 2018). Additionally, biomass of *T. obliquus* increased 3.5–24.8% when co-cultured with mixed bacterial cultures (Wang *et al.*, 2015). Dr. Ryan Powell, a former PhD student of the Hill laboratory, characterized the bacterial community associated with the HTB1 grown with air, flue gas, and 15% CO₂ (Powell, 2014). Dr. Powell found multiple spatial associations of bacteria and HTB1: symbionts were found as free-living cells, as single cells associated with individual algal cells, and in aggregates composed of multiple bacteria and HTB1 cells. Through 454 sequencing of 16S rRNA genes, HTB1 was found to be associated with a relatively simple bacterial community dominated by the phyla Proteobacteria and Bacteroidetes. The genus *Porphyrobacter* comprised 56% of the 16S rRNA gene sequences found in algal cultures grown with flue gas and 85% of the sequences found from algae grown in air (Powell, 2014). This same genus was found to be the second most abundant when HTB1 was grown with 10% CO₂ and ambient air. The similarities of my preliminary findings to those of Powell (2014) and others will be discussed further in Chapter 3, Section 3.5.1. The bacterial community associated with *N. oceanica* IMET1 was analyzed by members of the Hill laboratory using denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene-based clone library construction (Wang *et al.*, 2012). Through both methodologies, Alphaproteobacteria (48%) and Bacteroidetes (28%) dominated the culturable bacterial community. The same study showed that a particular bacterium (strain HW001), had a remarkable ability to aggregate and harvest several algae, including *N. oceanica* IMET1.

1.4.3 Sequencing bacterial communities and uncovering microbial dark matter

There are three main methods to characterize a microbiome, each having their own advantages and limitations. The most rudimentary method is to establish a ‘culturome’ by plating and identifying any microbes that can grow under laboratory settings. While this method is the most effective for obtaining bacterial stocks, it is labor intensive and only captures a small amount of true underlying diversity as most bacteria are unable to be cultivated by conventional approaches (Lagier *et al.*, 2018). Currently the most widely used method for microbiome characterization is amplicon based sequencing using the 16S rRNA gene for prokaryotes and the 18S rRNA gene and internal transcribed spacers (ITS) for eukaryotes (Y. X. Liu *et al.*, 2021). For bacterial community analysis, researchers must first choose primer sets that target a specific region of the 16S rRNA gene of which there are many (Abellan-Schneyder *et al.*, 2021). Current sequencing technology (often Illumina’s MiSeq, HiSeq 2500, or NovaSeq 6000 platforms) rely on a two-step polymerase chain reaction (PCR) to add barcodes and adaptors to each sample during library preparation and results in paired-end reads between 75-300 bases (de Muinck *et al.*, 2017). Illumina reads are characterized by their high accuracy, *ca.* 0.1% of base calling resulting in errors (Goodwin *et al.*, 2016). Amplicon sequencing has many advantages as it works efficiently on samples with low DNA concentrations and can target solely bacterial DNA inside of a eukaryotic host (Y. X. Liu *et al.*, 2021). However, amplicon-based sequencing produces reads that often only allow for genus-level resolution. It is also sensitive to PCR-based errors (known as sequencing chimeras) which can lead to both false positives and false negatives, the latter specifically for low abundant taxa (Y. X. Liu *et al.*, 2021). Metagenomic sequencing is the current gold standard as it does not rely on PCR and amplicon of certain gene regions. Instead, the reaction sequences all DNA within a sample regardless of its origin. This

method, performed mostly on instruments made by Oxford Nanopore and PacBio, has a considerably higher error rate compared to amplicon sequencing (*ca.* 5 to 15% of base calling resulting in errors). However, the substantial output and read length (10–12 thousand base pairs on average) allows for sequence coverage to compensate for errors (Loman *et al.*, 2015; Buck *et al.*, 2017). The major downsides to metagenomic sequencing are its price (10 times more expensive than amplicon sequencing at its minimum), its time-consuming bioinformatic analysis, and the inability to separate host-derived contamination (Y. X. Liu *et al.*, 2021). However, compared to 16S rRNA gene sequencing, metagenomic sequencing can recover complete bacterial genomes, providing a much higher resolution for taxonomic placement, and can provide full functional gene and metabolic profiles.

Metagenomics allows researchers to recover genomes from uncultivated microorganisms through direct sequencing of DNA from mixed environmental samples (Handelsman, 2004). These uncultivated organisms, known as “microbial dark matter”, were previously underestimated in their abundance because many are not recovered by amplicon based sequencing due to 16S rRNA gene divergence. It has been estimated that 50–100% of organisms sampled from dark matter phyla would evade detection in typical amplicon based protocols (Brown *et al.*, 2015). Over the past decade, metagenomic datasets increased from diverse global environments leading to the discovery that microbial dark matter had a shared evolutionary history (Rinke *et al.*, 2013; Brown *et al.*, 2015). From 201 single-cell genomes, Rinke *et al.* (2013) found a monophyletic relationship between the candidate phyla GN02, OD1, and OP11, which were renamed Gracilibacteria, Parcubacteria and Microgenomates, respectively. The authors then proposed grouping these phyla into a superphylum named “Patescibacteria” (Rinke *et al.*, 2013). Soon after, study of 789 additional draft genomes belonging to a monophyletic

lineage encompassing Patescibacteria argued that this group should be subdivided to including numerous additional phyla and the new name, Candidate Phyla Radiation (CPR) was proposed (Brown *et al.*, 2015). Both names, Patescibacteria and CPR, refer to the same group of bacteria and have been used interchangeably. I use the name Patescibacteria in this dissertation.

When phylogeny was constructed using alignment of the 16S rRNA gene, it was estimated that Patescibacteria comprise >15% of the entire bacterial domain (Brown *et al.*, 2015). Further, when phylogeny was constructed based on 120 concatenated proteins, it was suggested that the group comprised closer to 25% (Parks *et al.*, 2017). Consistent patterns have emerged across members of Patescibacteria including their cosmopolitan distribution (Brown *et al.*, 2015; Tian *et al.*, 2020), small cell size (Luef *et al.*, 2015; Proctor *et al.*, 2018), ultra-reduced genomes (Nelson and Stegen, 2015; Gios *et al.*, 2023), and highly limited metabolic capabilities (Brown *et al.*, 2015; Hug *et al.*, 2016). Most members of Patescibacteria are unable to biosynthesize most (or any) amino acids and all members lack complete gene pathways for electron transport chains and the TCA cycle, leading to the hypothesis that associations with more complex organisms may be obligatory to their survival (Nelson and Stegen, 2015; Chaudhari *et al.*, 2021). Limited metabolic function and predicted symbiotic lifestyles in Patescibacteria and lower phylogenetic ranks have been found to span large redox gradients from fully oxic to completely anoxic environments (Chaudhari *et al.*, 2021). Despite their gene absence, studies have reported Patescibacteria to be contributing members in various environments and bioremediation technologies (Probst *et al.*, 2018; Hu *et al.*, 2024). This dissertation recovered high quality and complete metagenomes from two Patescibacteria that were found to be dominant symbionts of both *T. obliquus* and *N. oceanica*.

1.5 Aims of this dissertation

- I. Visualize and quantify the two microalgae of this study *Tetradismus obliquus* strain HTB1 and *Nannochloropsis oceanica* strain IMET1 and their bacterial symbionts.**
 - a. Accomplished through brightfield microscopy, epifluorescent microscopy, scanning electron microscopy, and digital polymerase chain reaction.
- II. Characterize prokaryotic communities associated with cultures of *T. obliquus* strain HTB1 and *N. oceanica* strain IMET1**
 - a. Accomplished through sequencing and analysis of the 16S rRNA gene using Illumina MiSeq and QIIME2.
- III. Assemble and analyze high quality genomes from the most abundant members of the bacterial communities from each microalga.**
 - a. Accomplished through metagenomic sequencing and assembly of metagenomic assembled genomes (MAGs).
- IV. Assess potential functional contributions and metabolic pathways of bacterial communities associated with each microalga.**
 - a. Accomplished through annotation of functional genes within metagenomic sequencing pool and metabolic reconstruction of each MAG

AI disclosure: ChatGPT was used throughout bioinformatic analysis for program download instructions, code error interpretation, and troubleshooting.

Chapter 2: Spatial associations between microalgae and their bacterial symbionts

2.1 Abstract

An often-overlooked factor in applied phycology is the significance of symbiotic associations between microalgae and bacteria, including the wide range of benefits that bacterial cells can provide to their hosts. Microalgae transform fixed carbon dioxide into organic carbon compounds, which are utilized by bacteria who then secrete vitamins, siderophores, and remineralized nutrients in return. While the spatial associations between microalgae and bacteria are well studied in nature, less attention has been paid to industrial microalgal cultures grown for biomass production. In this chapter, I used three forms of microscopy to visualize both *Tetrademus obliquus* HTB1, *Nannochloropsis oceanica* IMET1, and both algae's bacterial consortia. Light microscopy revealed size and morphology of the algal cells. DAPI staining paired with epifluorescent microscopy allowed for the distinction between each cell type. Scanning electron microscopy (SEM) provided higher resolution to show that cultures of the two microalgae contain bacterial symbionts that are both closely associated (attached to the algal cell) and free-living. Digital polymerase chain reaction (dPCR) was used to quantify microalgal and bacterial cells. For IMET1, the ratio of microalgal cells to bacterial cells was found to be approx. 1:5. This ratio was found in the microalgal cultures that were filtered onto 0.45 μm filters, retaining bacteria considered to be closely associated symbionts. The ratio was also found in filtrate that passed through 0.45 μm filters and was concentrated on 0.22 μm filters, capturing free-living symbionts that were not tightly associated with the microalgal cells. For HTB1, the ratio of microalgal cells to bacterial cells to was found to be approx. was 2.5:1. This ratio held consistent in the 0.45 μm fraction for one replicate experiment while almost no algal or bacterial genes were amplified from the 0.22 μm fraction. This chapter provides a visual framework for

better understanding the types of associations that *N. oceanica* and *T. obliquus* share with their bacterial symbionts.

2.2 Introduction

2.2.1 Endosymbiotic associations of microalgae and bacteria

Interactions between microalgae and bacteria are important in aquatic ecology and biogeochemistry. Their shared history suggests they have coexisted since the early stages of evolution, and several studies have shown that various algal and bacterial species affect each other's survival, physiology, and growth (Ramanan *et al.*, 2016). Endosymbiosis between microalgae and bacteria is a particularly significant relationship. A primary endosymbiosis event led to the formation of eukaryotes when a heterotrophic eukaryotic ancestor engulfed a cyanobacterium or cyanobacterial-like prokaryote (as reviewed by Bhattacharya and Medlin, 1995). The cyanobacterium was then retained and served as an organelle, thus enabling photosynthesis in a eukaryote (Gould *et al.*, 2008). Yoon *et al.*, 2004 estimates this event most likely took place more than 1,500 million years ago based on fossil record. Endosymbiotic bacteria are still prevalent in present day microalgae. Some examples include an endosymbiont of the *Rickettsiaceae* family detected by transmission electron microscopy (TEM) inside of *Volvox carteri* (Kawafune *et al.*, 2014) and *Bacillus tequilensis*, an endosymbiont of *Haematococcus lacustris* found through fluorescence *in situ* hybridization (FISH) (Jeon *et al.*, 2023). In the latter symbiosis, *B. tequilensis* was shown to improve growth, chlorophyll content, and astaxanthin content in *H. lacustris*. In two recent studies, several endophytic bacteria (endosymbionts of plant cells) were cultured and shown to contain several genes relating to plant growth promotion (PGP); one bacterium in particular, *Bacillus paralicheniformis* PPE102, was then co-cultured with the microalga *Micractinium* sp. GA001 and positively affected growth (Sonowal, Ahmed, *et al.*, 2022; Sonowal, Palani, *et al.*, 2022). The positive contributions of

bacteria to their microalgal hosts are also well-characterized outside of endosymbiotic interactions, for instance in the algal “phycosphere.”

2.2.2 Bacterial symbionts residing in the microalgal “phycosphere”

The phycosphere, a term first coined in 1972, is the space around a microalgal cell in which extracellular products are exuded and utilized by bacteria (Bell and Mitchell, 1972). The phycosphere is considered a hotspot for symbiosis due to high concentrations of fixed organic carbon compounds that stimulate bacterial growth (Seymour *et al.*, 2017). The bacteria residing within the phycosphere could be commensals, scavenging carbon while offering no benefit in return (Ramanan *et al.*, 2016). However, the bulk of research tends to suggest mutualism, where bacteria provide remineralized nutrients and vitamins in exchange for carbon exudates. In nature, algal bloom dynamics are highly temporal and one study found attachment of bacteria to the phycosphere varied based on the stage of the bloom (Mayali *et al.*, 2011). In the laboratory, the microbial community in the phycosphere remains relatively consistent and can be manipulated to increase algal yield and performance. For example, one study cultured several isolates from the phycosphere of *Chlorella vulgaris* and observed enhanced algal growth, flocculation, and lipid content when the bacterial consortium was reintroduced into a coculture (Cho *et al.*, 2015). Another study found that in *Phaeodactylum tricornutum*, 92%–98% of microalgal cells were reported to have attached bacteria with some cells having up to eight bacteria attached; *P. tricornutum* fixed 64% more carbon with attached bacteria compared to axenic microalgal cells (Samo *et al.*, 2018). In the same study, 42%–63% of *Nannochloropsis salina* had attached symbionts and bacterial attachment had a negative effect on carbon fixation rates. The phycosphere has become a widely researched microhabitat for bacteria and it has become clear

that although the associations are not always species-specific, each organism depends on the other for certain key metabolites (Grossart *et al.*, 2005; Ramanan *et al.*, 2015; Wei *et al.*, 2023).

2.2.3 Free-living bacterial symbionts of microalgae

Symbiosis between microalgae and bacteria does not necessarily depend on proximity. Considering the physical instability of aquatic environments (compared to that of motionless soil or rhizosphere environments), cells often exist in free-living states. While endosymbiotic or attached bacteria may have a spatial advantage for algal exudates, free-living bacteria may benefit from acquiring highly soluble compounds from a greater distance and over greater time periods (Seymour *et al.*, 2017). Several studies have found that communities of free-living and closely associated bacteria are taxonomically distinct (DeLong *et al.*, 1993; Fandino *et al.*, 2001; Grossart *et al.*, 2005). Further, free-living assemblages have been found to promote microalgal growth, for instance in *Chlamydomonas reinhardtii*, *C. vulgaris*, and *Euglena gracilis*. Each culture had a higher dry weight and chlorophyll content when supplemented with indigenous bacteria from wastewater effluent (Toyama *et al.*, 2018). One mechanism for increased algal biomass is complex nutrient and hormone exchange between free-living cells. Amin *et al.*, 2015 found that secretion of the hormone indole-3-acetic acid (IAA) by a free-living *Sulfitobacter* sp. promoted cell division of *Pseudonitzschia multiseries*. The production of IAA by bacteria is stimulated by algal-excreted tryptophan and this mutualistic exchange is well documented (Lin *et al.*, 2022). The role of various interactions between microalgae and bacteria within coculture systems is key in enhancing carbon fixation rates and developing more efficient technologies.

2.2.4 Overview of this chapter

This chapter provides both qualitative and quantitative data to understand the spatial relationship of microalgae and bacteria in our two systems. Cultures of *T. obliquus* HTB1 and *N. oceanica* IMET1 were visualized by light microscopy, epifluorescent microscopy, and SEM. Microalgal cells are easily visualized by epifluorescent microscopy due to the strong autofluorescence of chlorophyll and accessory pigments (Carreira *et al.*, 2015). Bacterial symbionts can be visualized by first staining the cells with 4',6-diamidino-2-phenylindole (DAPI). By precisely defining excitation and emission spectra of microalgal autofluorescence and DAPI, both cells can be visualized at once. SEM was used for higher magnification and spatial understanding of microalgal/bacterial associations within our system. Once satisfactory images were obtained, dPCR was used to independently quantify the ratio of microalgae to bacteria for HTB1 and IMET1.

2.3 Methods

2.3.1 Light microscopy, epifluorescent microscopy, and scanning electron microscopy (SEM)

For light microscopy, microalgal cultures were diluted 10^0 – 10^{-4} , pipetted directly into 96-well plates, and visualized with a Keyence BZX800 microscope. For epifluorescent microscopy, cells were washed once with 1X phosphate buffer solution (PBS), fixed 1:1 in 4% formaldehyde for 1 hour, and washed again. The pellet was resuspended in 1.2 μ M 4',6-diamidino-2-phenylindole (DAPI) and left to soak in the dark for 30 minutes. Cells were filtered onto 0.1 μ m black polycarbonate filters (GVS Life Sciences), placed on microscope slides, treated with Diamond Antifade Mountant (ThermoFisher) and sealed with a coverslip. Slides were visualized with a Nikon W1 spinning disk microscope at the Confocal Microscopy Facility at the University of Maryland School of Medicine. In total eight microscopes were used for this dissertation (Table 2.1). Microalgal autofluorescence signal was detected at 488 nm (excitation)/ 500-550 nm (emission) and DAPI was detected at 405 nm (excitation)/ 430-480 nm (emission). Excitation and emission spectra and peaks for chlorophyll, microalgal accessory pigments, and nucleic acid stains are shown in Table 2.2.

To prepare samples for SEM, a custom methodology was developed. Microalgae-bacteria suspension (2 ml) was collected and centrifuged at 10,000 g for 3 minutes. The pellet was washed once with 1X PBS to ensure the removal of any residual medium. Cells were fixed with 2.5% glutaraldehyde and 4% formaldehyde for 2 hours (Pagnussat *et al.*, 2020) and subsequently washed twice with 1X PBS. Glass coverslips were washed with acetone and coated three times with poly-L-lysine (molecular weight 70,000–150,000). Coverslips (size 12 mm, no 1 thickness) were submerged into the fixed microalgae-bacteria suspension for 30 minutes at room

temperature followed by an ethanol gradient (30, 50, 70, 80, 90, and 100% 3x) for 10 minutes each step. Immediately following removal from the 100% ethanol, 20 μ l of HMDS (1,1,1,3,3,3-hexamethyldisilazane $\geq$ 97.0%) was pipetted directly onto the coverslips. Coverslips were left to dry for 2 minutes and a second coat of 20 μ l of HMDS was applied. Coverslips were then mounted onto stubs, sputtered with gold-palladium in a sputter coater (Cressington 108 Manual), and imaged using a FEI Nova NanoSEM 450 at the University of Maryland Baltimore County.

Table 2.1 Microscopes used to obtain micrographs of microalgae and their bacterial symbionts.

Microscope Name	Location	Type of microscopy	Outcome
Keyence BZX800	IMET (temporary demo)	Light and LED fluorescence	Success
Common equipment	IMET common use	Epifluorescent	Issue with 60X objective
Tracy	Tracy Laboratory IMET	Epifluorescent	Condensation in camera lens
Leica	IMET common use	Laser Confocal-Epifluorescent	Unable to separate fluorescence signals
Nikon AX/AXR with NSPARC	University of Maryland Baltimore	Laser Confocal-Epifluorescent	Unable to separate fluorescence signals
Nikon Spinning Disk W1	University of Maryland Baltimore	Laser Confocal-Epifluorescent	Success
FEI Nova NanoSEM 450	University of Maryland Baltimore County	SEM	Success

Table 2.2 Excitation and emission spectra in nanometers (nm) to detect autofluorescence in microalgae as well as common stains for nucleic acids.

Microalgal autofluorescence	Excitation (nm)	Emission (nm)
Chlorophyll a	430 & 662	640 - 680
Chlorophyll b	453 & 642	640 - 680
Accessory pigments	450 - 650	550 - 700
Nucleic acid stains	Excitation (nm)	Emission (nm)
DAPI	358 - 365	430 - 450
SYBR Gold	495	537
SYBR Green	490 - 495	520 - 530

2.3.2 Digital PCR (dPCR)

Microalgal cultures were normalized to an optical density (absorbance at 750 nm) of 0.8 and processed in two ways. First, 40 ml of culture was filtered onto a 0.22 μ m cellulose acetate filter (Advantec). This size fraction captures both microalgal cells and all bacterial symbionts. Second, additional samples were size fractionated: 30 ml was filtered onto a 0.45 μ m cellulose acetate filter and flowthrough was filtered onto a 0.22 μ m filter. DNA from all filters was extracted using the Powerwater DNA extraction kit (Qiagen). Digital polymerase chain reaction (dPCR) was used to count gene copies of the eukaryotic 18S rRNA gene and bacterial 16S rRNA gene, with methods modified from Yu *et al.*, 2022. The QIAcuity EvaGreen PCR Kit (Qiagen) was used to amplify 18S rRNA gene fragments using the eukaryote-specific 528F/706R primer pair: 528-F (5'-GCGGTAATTCCAGCTCCAA-3'); 706-R (5'-AATCCRAGAATTTTCACCTCT-3') (Elwood *et al.*, 1985; Zhang *et al.*, 2018). The QIAcuity Probe PCR Kit was used to amplify 16S rRNA gene fragments using the bacterial-specific BAC primer/probe set: BAC338F (ACTCC TACGG GAGGC AG); BAC805R (GACTA CCAGG GTATC TAATC C); Probe-56FAM 3TAMRA BAC516F (Taqman) (TGCCA GCAGC CGCGG TAATA C) (Yu *et al.*,

2005). A non-template control (DI water) was included in each run. The reaction and primer/probe mixes were prepared as per manufacturer's instructions. The prepared mixtures were loaded onto a QIAcuity 8.5k 96-well Nanoplate and analyzed in a QIAcuity ONE machine with the following cycling conditions: 95°C for 2 minutes, 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds, and a final step of 35°C for 5 minutes. Triplicate reactions were run for each sample and error bars were calculated with standard error.

2.3.3 Algal measurements using optical density and cell counting

For optical density, absorbance of samples was measured by using a Nanodrop 2000c Spectrophotometer (Thermo Fisher Scientific) at 750 nm (OD₇₅₀). For cell counting, microalgal cultures were diluted 10^{-1} – 10^{-2} in Isoflow sheath fluid (Beckman Coulter) and placed into a Multisizer 4e Coulter Counter (Beckman Coulter) which works by counting cells as they pass through an electromagnetic field; from the disruption, the machine is able to count and tell the shape and size of particles. (Bull *et al.*, 1965). The size of aperture can measure 2-60% of particle size. Because *N. oceanica* and *T. obliquus* cells are 3-8 µm, a 70 µm aperture was used.

2.4 Results

2.4.1 Light microscopy shows microalgal size and morphology

Brightfield microscopy revealed size and morphology of *T. obliquus* HTB1 (Figure 2.1) and *N. oceanica* IMET1 cells (Figure 2.2). HTB1 was prepared at various dilutions and imaged at various magnifications (Figure 2.1). HTB1 cells are bright green, averaged 5.3 μm in diameter (Figure 2.3), and are often oval or lemon shaped. The bottom two panels of Figure 2.1 show HTB1 cells visualized with 60X and 100X objectives and reveals the most morphological information, including the alga's central vacuole (orange arrow).

IMET1 cells are smaller in size, averaged 2.6 μm in diameter (Figure 2.3), and are circular in shape (Figure 2.2). IMET1 cells are lighter in color than HTB1, which is also seen with the naked eye when preparing liquid cultures. In general, IMET1 cells are more difficult to visualize with light microscopy compared to HTB1. This is in part due to their size, but also the visual planes in which cells settle in a 96-well plate and under coverslips (Figure 2.2). The diameter of each alga was confirmed using a Coulter Counter and shown in Figure 2.3.

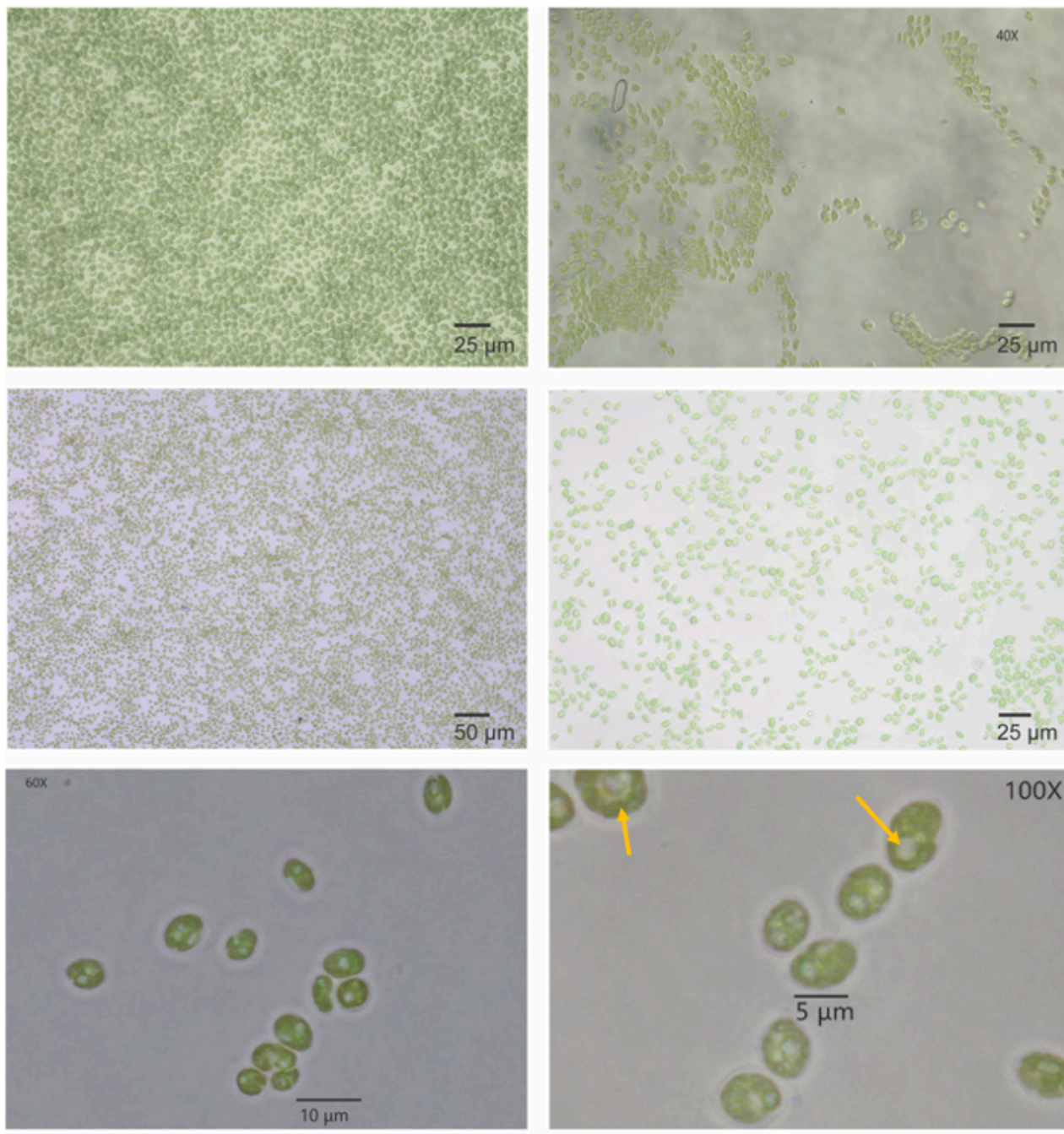


Figure 2.1 Micrographs of *Tetradismus obliquus* strain HTB1 taken by light microscopy at various dilutions and magnifications

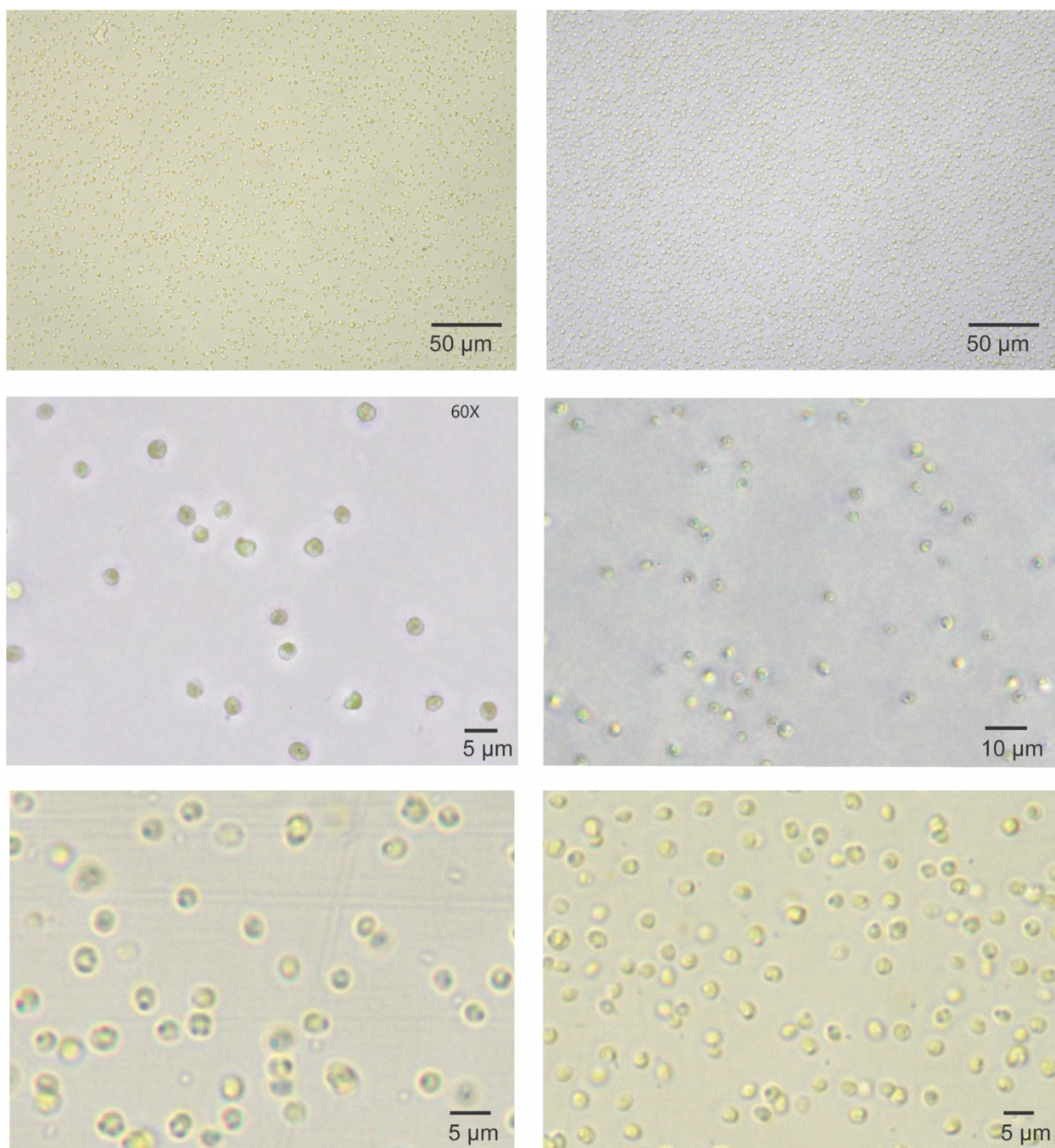


Figure 2.2 Micrographs of *Nannochloropsis oceanica* strain IMET1 taken by light microscopy at various dilutions and magnifications.

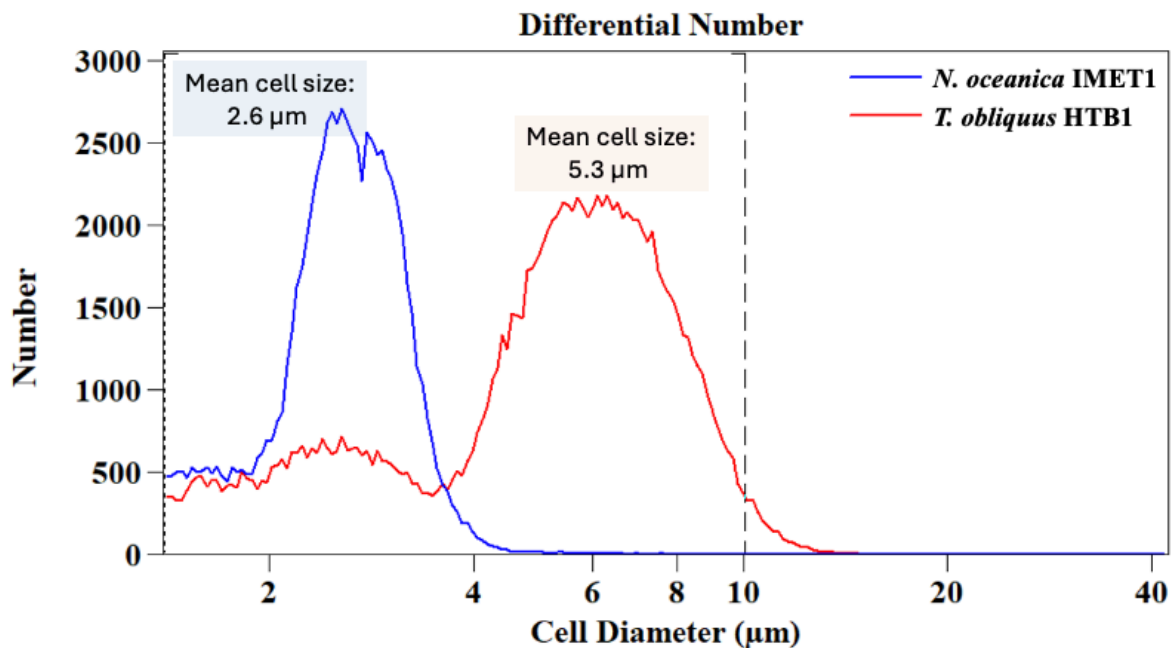


Figure 2.3 Size distribution of *N. oceanica* IMET1 (blue) and *T. obliquus* HTB1 (red) using a Coulter Counter. Peaks indicate that IMET cells have a mean diameter of 2.6 μm and HTB1 cells have a diameter mean of 5.3 μm.

2.4.2 Epifluorescent microscopy

Microalgal autofluorescence is shown in Figure 2.4. Cultures of unstained *T. obliquus* cultures were visualized with an epifluorescent microscope. In Figure 2.4, both micrographs are identical apart from the light source. The left panel shows a light microscopy image, and in the right panel, the sample was excited by a laser (FITC channel at 488 nm), demonstrating microalgal autofluorescence without the use of a fluorescent dye. The same phenomenon occurs with *N. oceanica* (Figure 2.7 panel A).

DAPI, SYBR Gold, and SYBR Green are fluorescent dyes that stain nucleic acid and can be used to visualize bacteria. DAPI was ultimately chosen because of its membrane impermeability (Yoshinari *et al.*, 2024); both HTB1 and IMET1 took up DAPI less than SYBR

Gold and SYBR Green. This is important for distinguishing algal cells from bacterial cells.

Figure 2.5 shows identical micrographs, the left panel containing HTB1 culture stained with DAPI and visualized with visible light and the right panel excited by the DAPI channel (405 nm). Some HTB1 cells take up DAPI as can be seen by their fluorescent nucleus (yellow arrows). IMET1 took up more DAPI than HTB1 (Figure 2.6, yellow arrows). However, it is clear that only a small fraction of IMET1 cells took up DAPI when comparing the right and left panels of Figure 2.6. When algal cultures containing bacterial symbionts are stained with DAPI and excited with two lasers using a confocal microscope, autofluorescence and DAPI fluorescence can be digitally assigned different colors. Consequently, both microalgae were imaged with their bacterial symbionts (Figures 2.7 and 2.8). During a stage in which a suitable microscope camera was not available, I used my iPhone camera through the Leica confocal microscope eyepiece to visualize both IMET and HTB1 with their DAPI-stained bacterial symbionts. Through this method, I was unable to properly assess size with a scale bar, however the photos clearly demonstrate both algae have bacterial communities with diverse morphologies (Appendix, Figures A2.1 and A2.2).

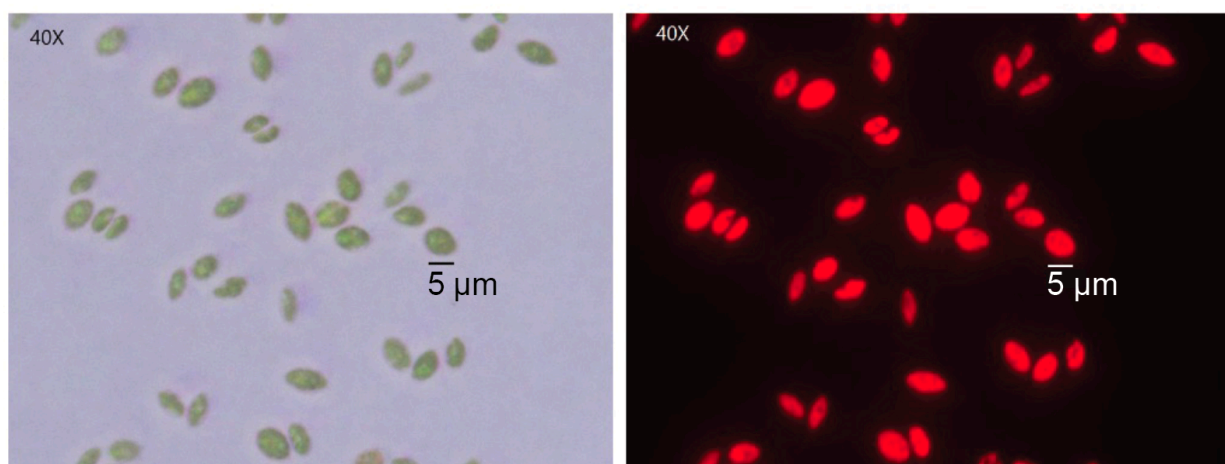


Figure 2.4 Unstained *T. obliquus* HTB1 cells visualized with standard light microscopy (left) and excited by laser showing microalgal autofluorescence (right).

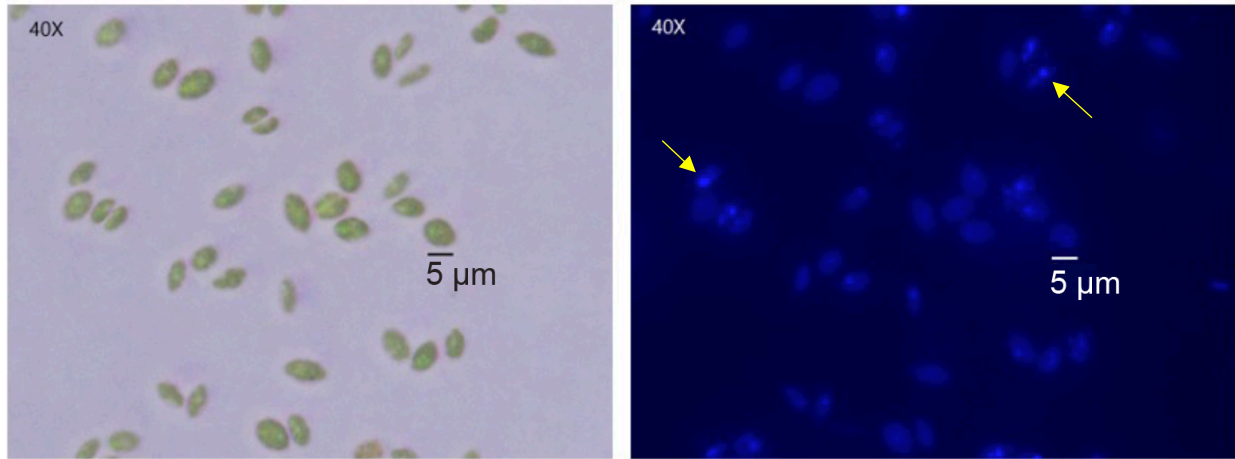


Figure 2.5 *T. obliquus* HTB1 cells stained with DAPI, a membrane-impermeable nucleic acid stain. Left panel shows cells visualized with standard light and right panel shows same cells excited by laser (405 nm excitation/ 430-480 nm emission). Uptake of DAPI by HTB1 cells is infrequent as stained nucleic acid is shown with yellow arrows.

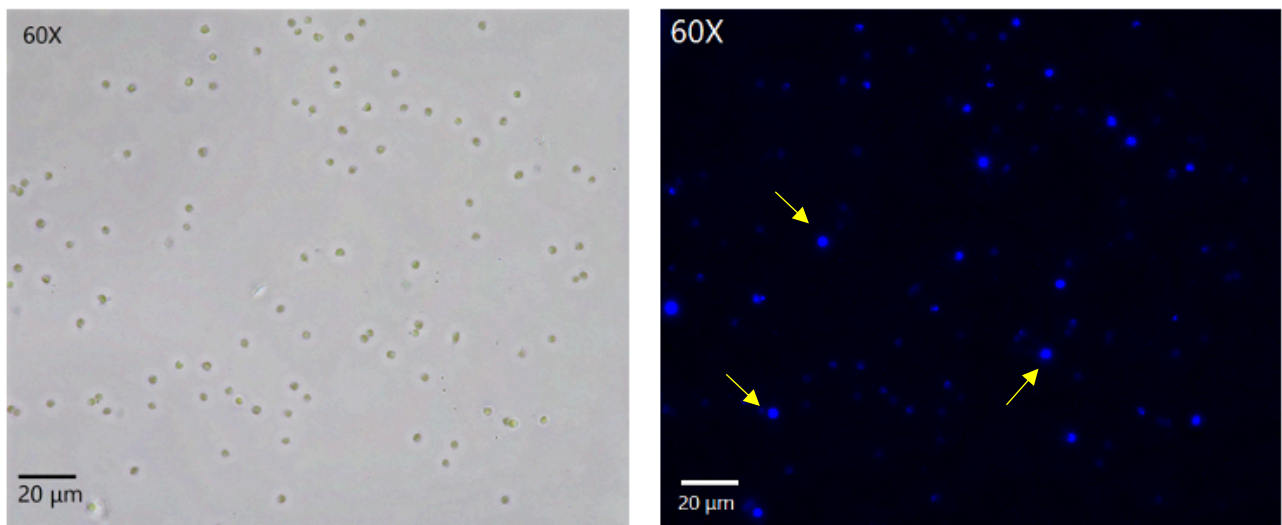


Figure 2.6 *N. oceanica* IMET1 cells stained with DAPI visualized with standard light microscopy (left) and by laser (405 nm excitation/ 430-480 nm emission). Uptake of DAPI by IMET1 cells is more frequent as stained nucleic acid is shown with yellow arrows.

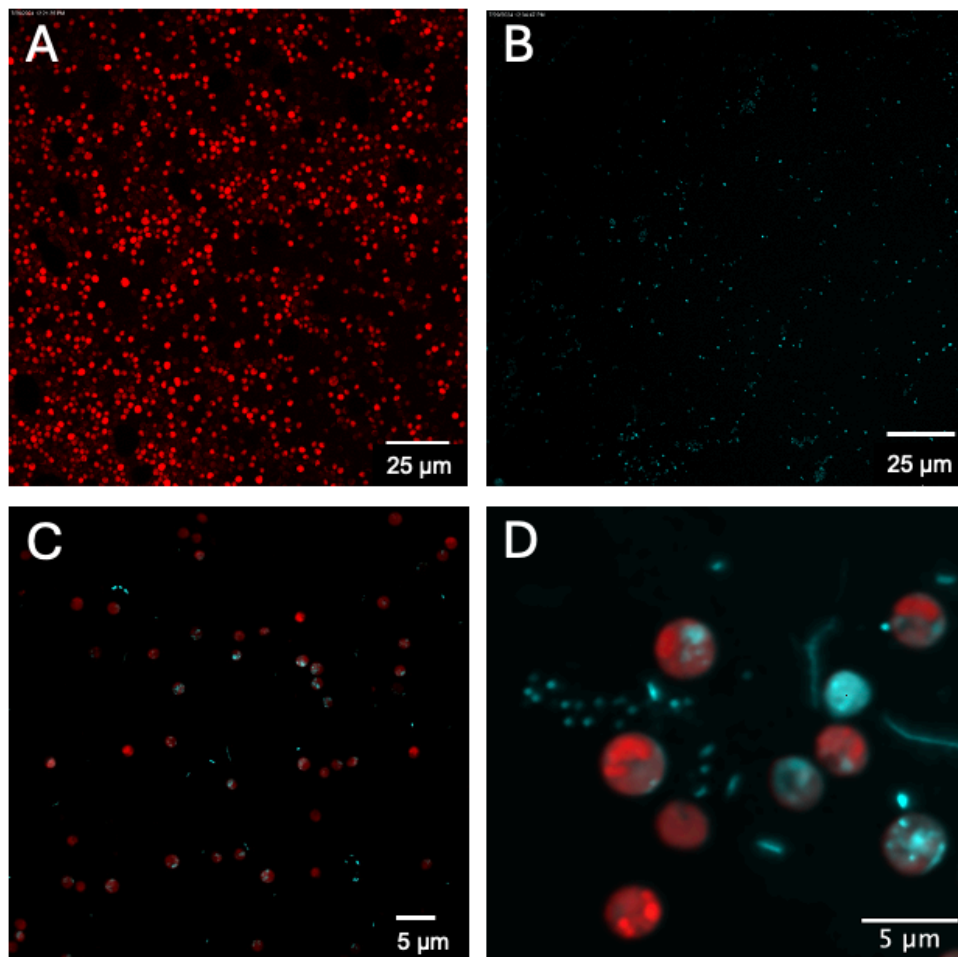


Figure 2.7 (A) Autofluorescence control: unstained IMET1. (B) Bacterial control: pure culture of bacteria stained with DAPI. (C & D) IMET1 and bacterial symbionts. Autofluorescence signal was detected at 488 nm (excitation)/ 500-550 nm (emission) and DAP

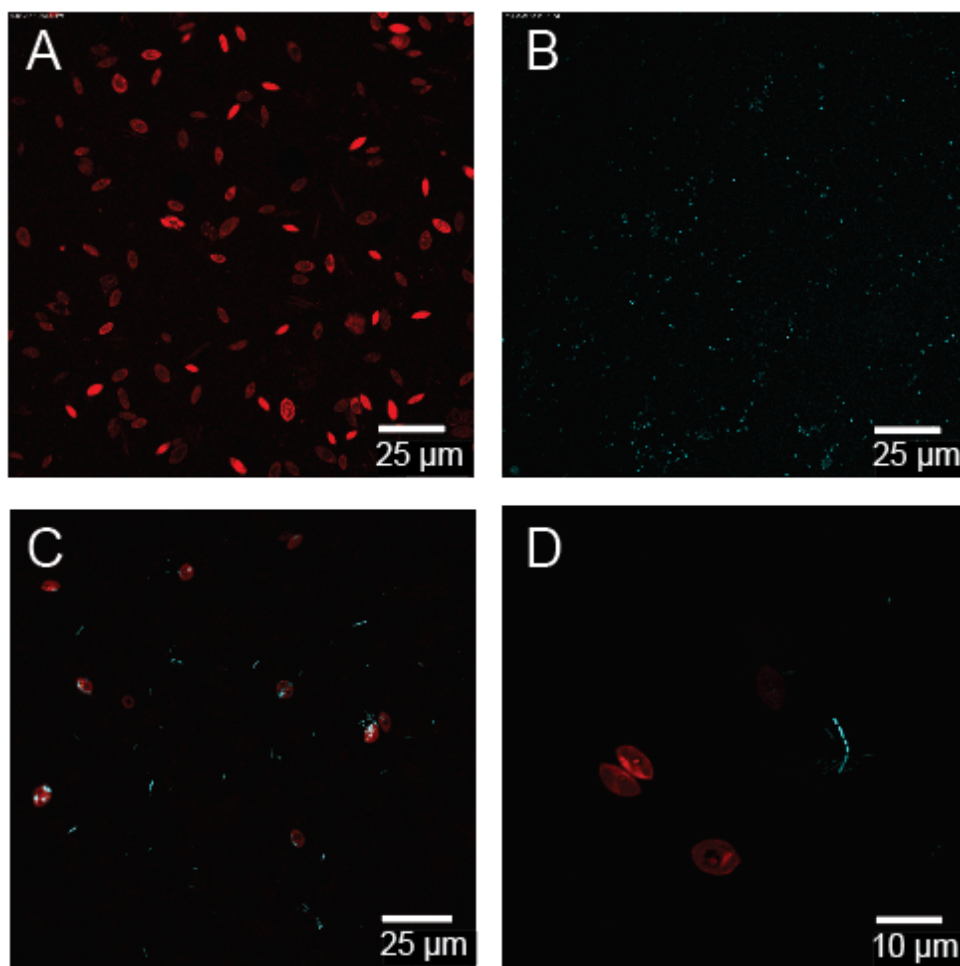


Figure 2.8 (A) Autofluorescence control: unstained HTB1. (B) Bacterial control: bacteria stained with DAPI. (C & D) HTB1 and bacterial symbionts. Autofluorescence signal was detected at 488 nm (excitation)/ 500-550 nm (emission) and DAPI was detected at 405 nm (excitation)/430-480 nm (emission). Slides were visualized with a Nikon W1 spinning disk microscope at the Confocal Microscopy Facility at the University of Maryland School of Medicine.

2.4.3 Scanning electron microscopy

SEM allows for higher magnification and resolution (5,000X – 19,000X) compared to brightfield and epifluorescent microscopy (200X – 1000X). This allows for better visualization of size and morphology of IMET1 (Figure 2.9) and HTB1 (Figure 2.10). SEM confirms IMET1 as spherical and 2-3 μm in size and HTB1 as 5-6 μm in size, and lemon shaped. Both IMET1 and HTB1 were visualized with both closely associated and free-living bacterial symbionts (Figures 2.11 – 2.14). Symbionts were considered as closely associated if touching or directly next to the microalgae. Symbionts of IMET1 were bacillus shaped, coccus shaped and formed chains often longer than the algal cell (Figure 2.11). The bacterial chains were also visualized by epifluorescent microscopy (Figure 2.7, panel D). A spirochete was imaged as a free-living bacteria associated with IMET1 (Figure 2.12, bottom panel). This individual is perhaps *Leptonema* sp. or another member of the Leptospiraceae family, consistent with these 16S rRNA gene sequences being found through microbiome analysis, discussed in Chapter 3 (Figures 3.9 and 3.12) Bacterial symbionts of HTB1 were coccus-shaped and bacillus-shaped, and were visualized on top of the microalgal cell (Figure 2.13) as well as free-living (Figure 2.14).

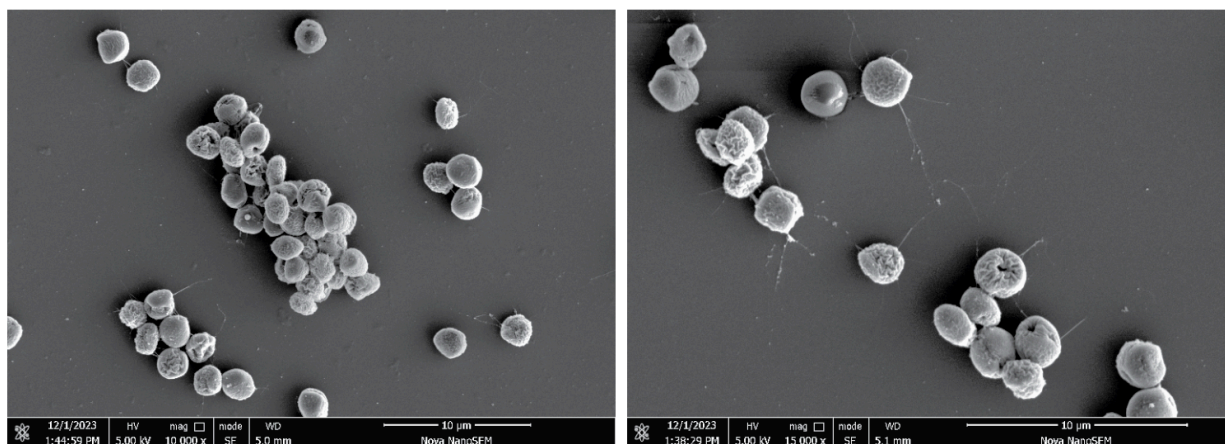


Figure 2.9 SEM micrographs of *N. oceanica* IMET1 imaged using a FEI Nova NanoSEM 450 at the University of Maryland Baltimore County.

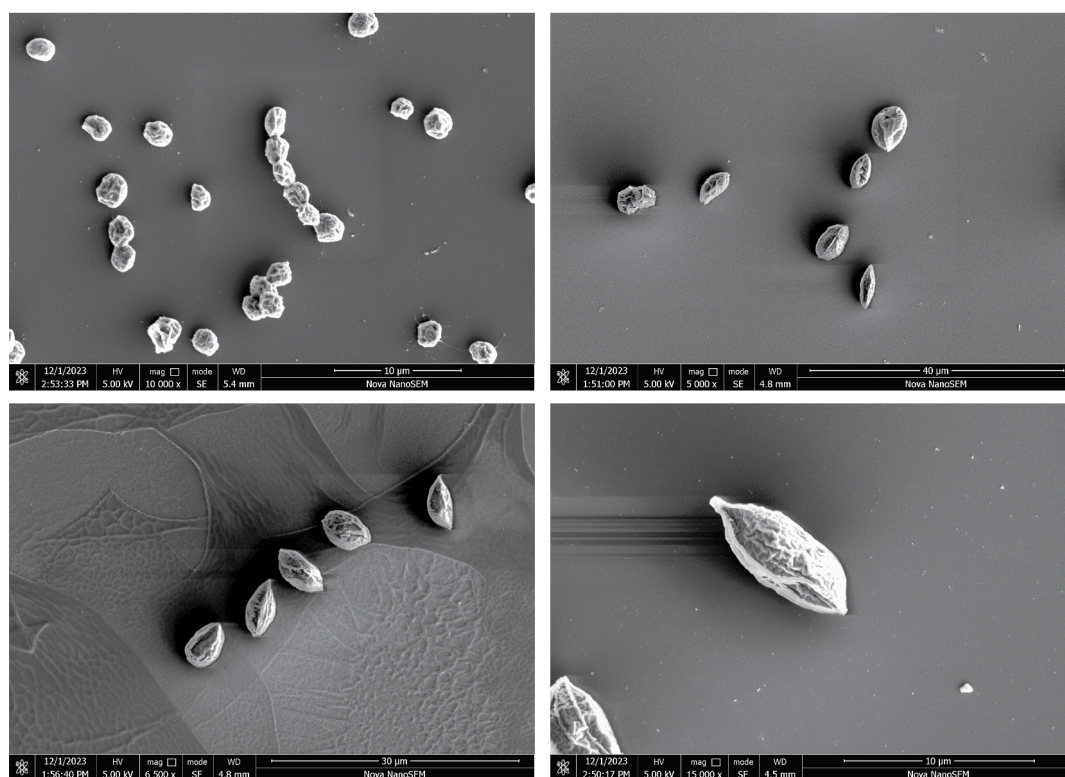


Figure 2.10 SEM micrographs of *T. obliquus* HTB1 imaged using a FEI Nova NanoSEM 450 at the University of Maryland Baltimore County.

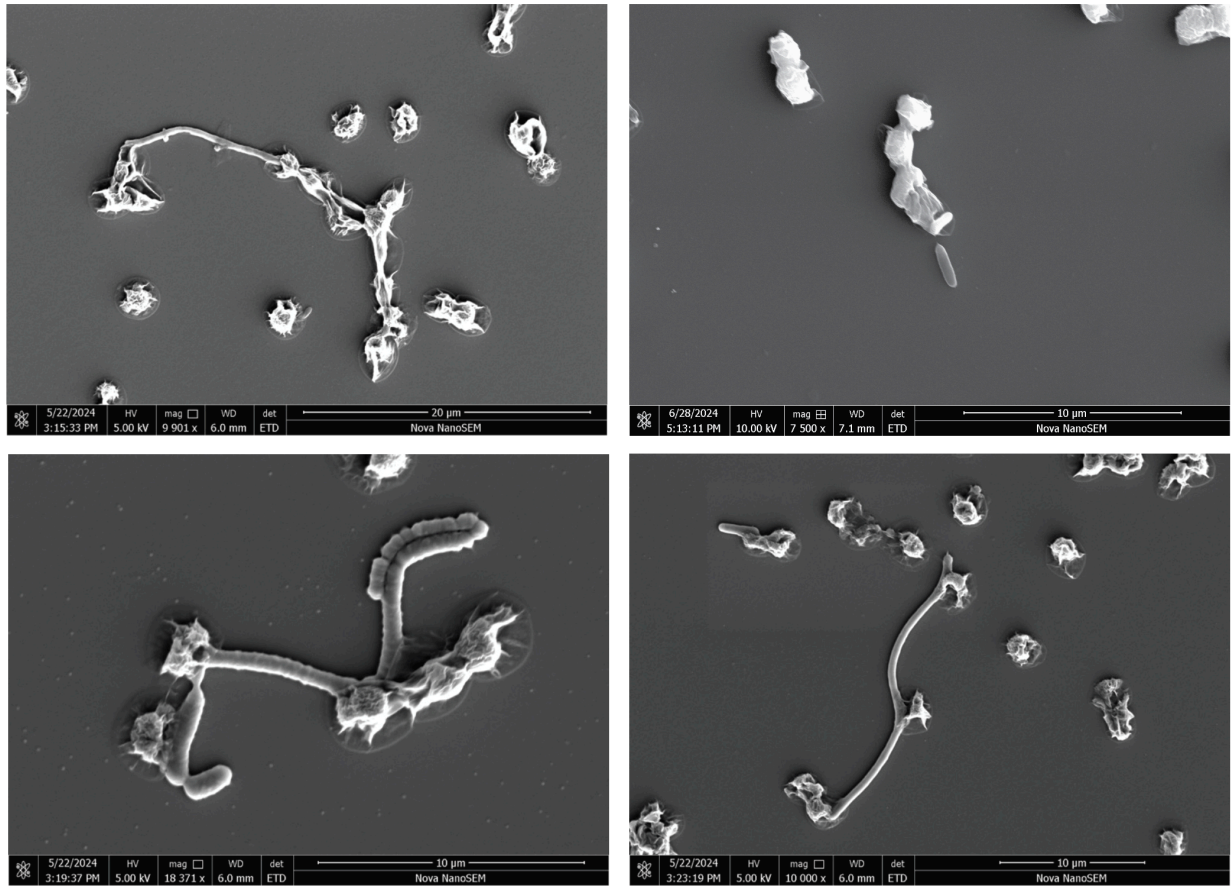


Figure 2.11 SEM micrographs of *N. oceanica* IMET1 and closely associated bacterial symbionts

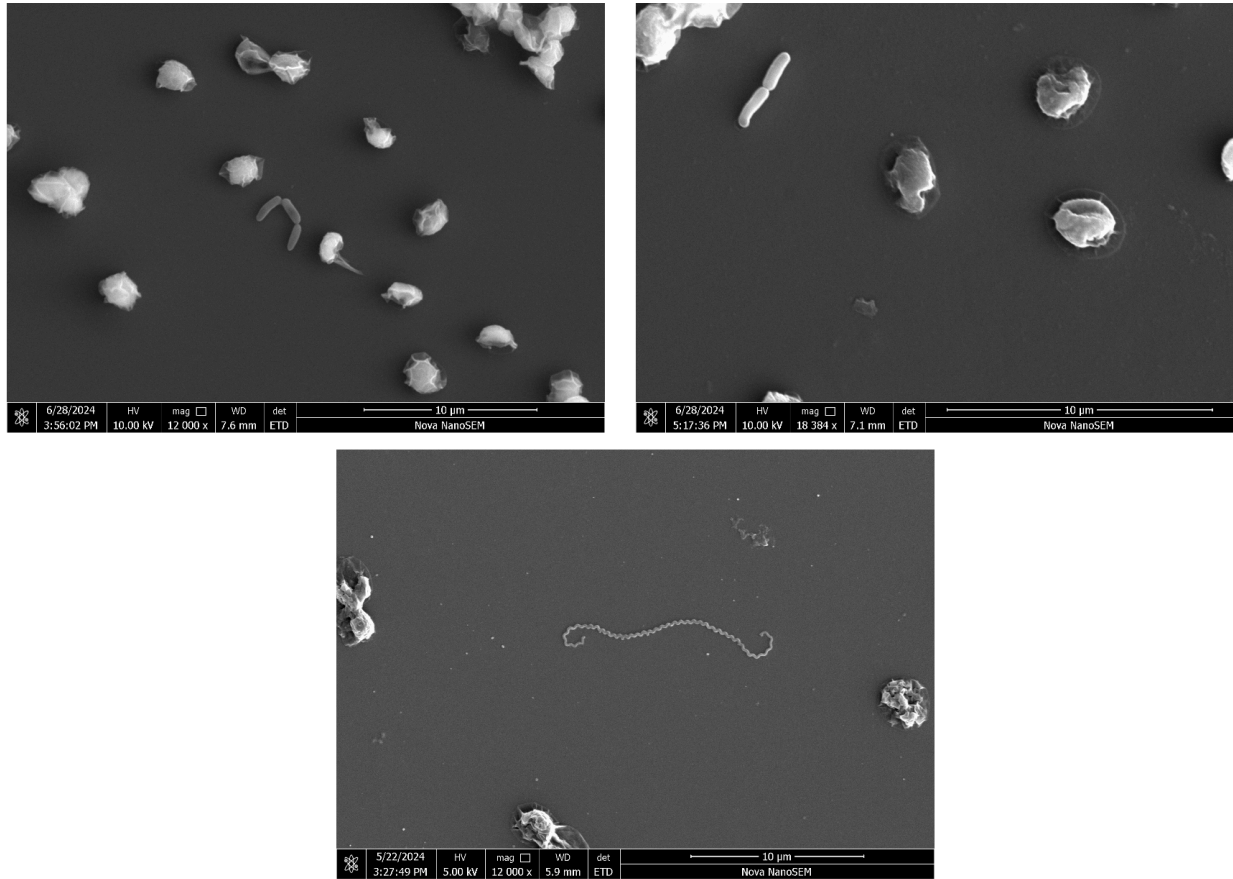


Figure 2.12 SEM micrographs of *N. oceanica* IMET1 and free-living bacterial symbionts.

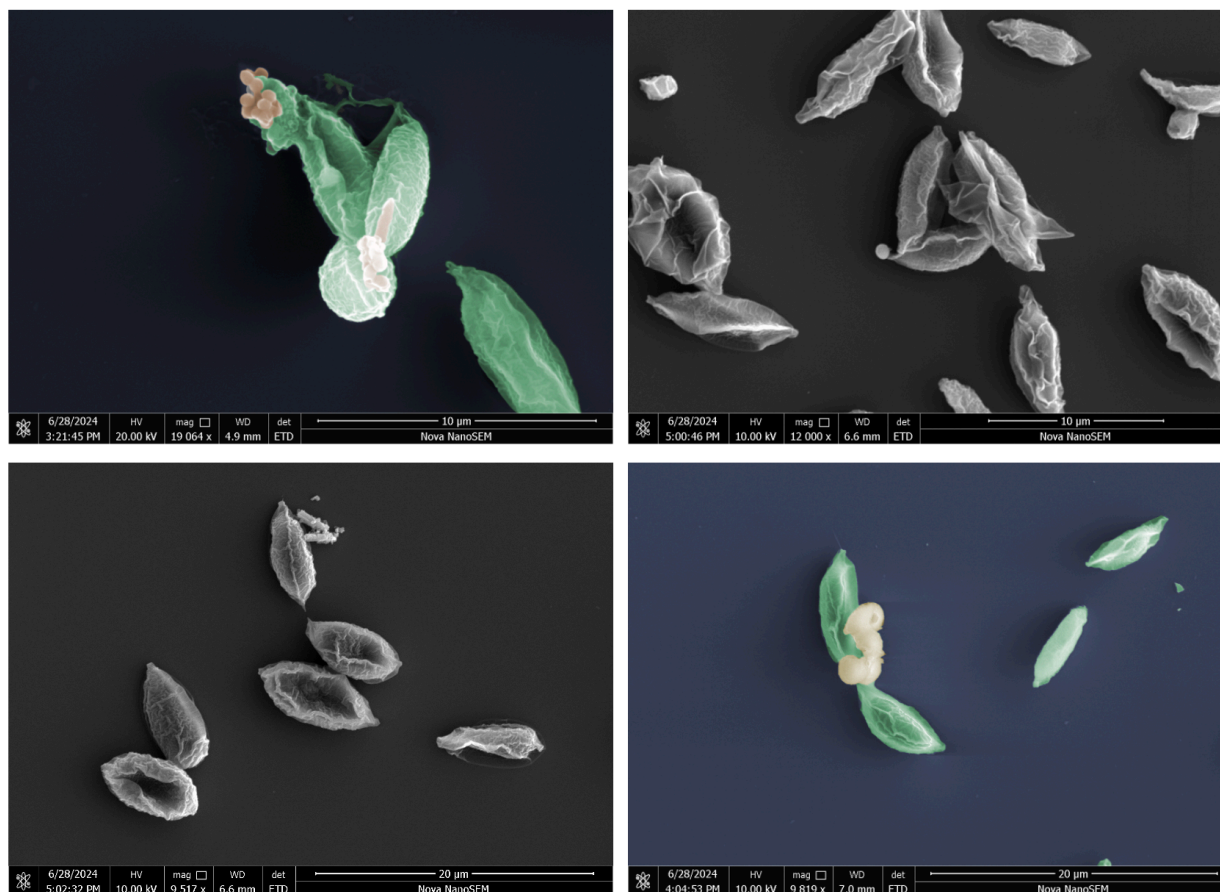
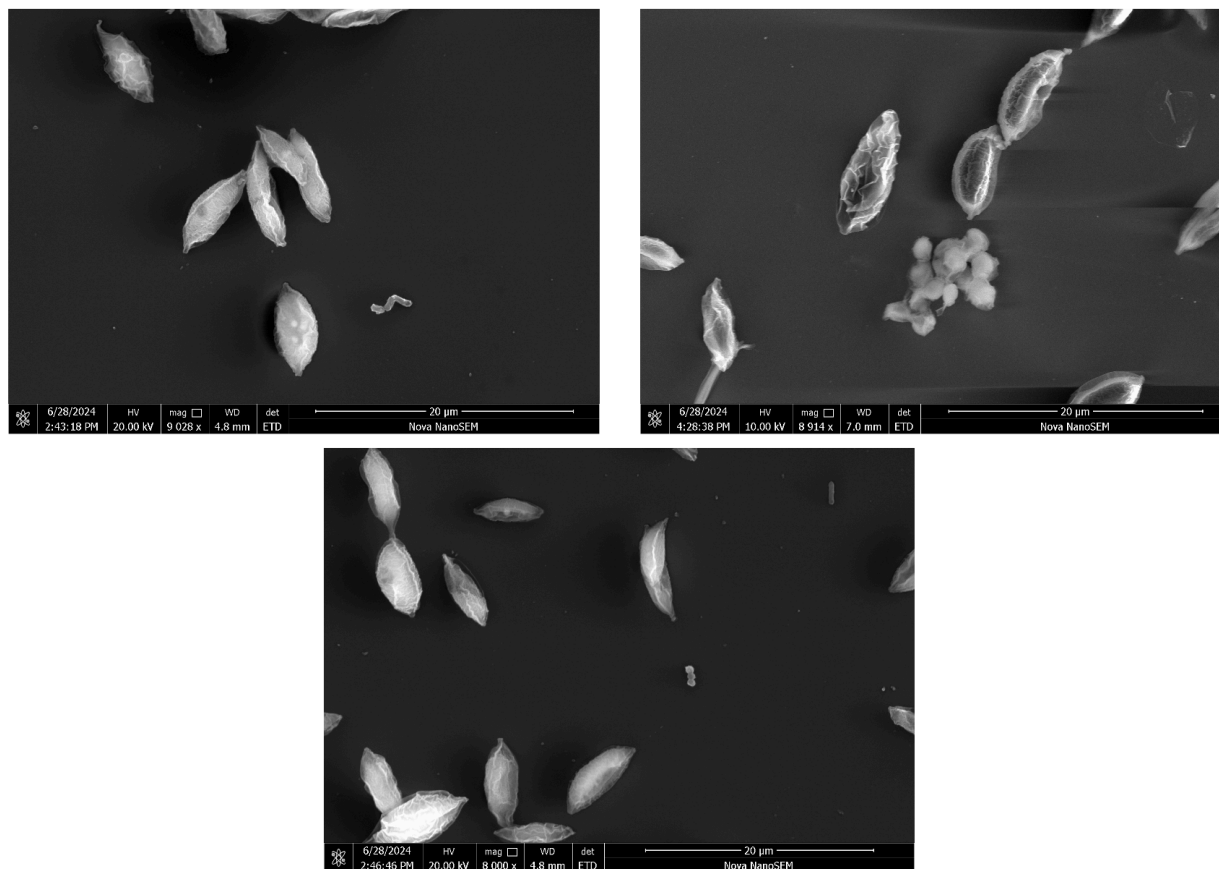


Figure 2.13 SEM micrographs of *T. obliquus* HTB1 and closely associated bacterial symbionts.

The top left and bottom right panels have been pseudo-colored, with HTB1 in green and bacteria in orange.



2.14 SEM micrographs of *T. obliquus* HTB1 and free-living bacterial symbionts.

2.4.4 Establishing ratios of microalgal cells:bacterial cells using digital PCR (dPCR)

dPCR was used to amplify and count the 18S rRNA gene and a bacterial-specific region of the 16S rRNA gene to determine the quantitative relationship between each microalga and their bacterial symbionts. For HTB1, the ratio of microalgal cells to bacterial cells was 2.5:1 when the cultures were not fractionated by size (Figure 2.15). This ratio held consistent in the 0.45 µm fraction for one experiment conducted in July 2024 but increased to 4.6:1 in a replicate experiment in August 2024 (Figure 2.16). Significantly fewer gene copies for microalgae and bacteria cells were detected in the 0.22 µm fraction for either experiment (Figure 2.16). For IMET1, the ratio of microalgal cells to bacterial cells was 1:5 (Figure 2.17). This ratio held

consistent regardless of whether the microalgal cultures were filtered onto a 0.45 μm filter or onto 0.22 μm (Figure 2.18).

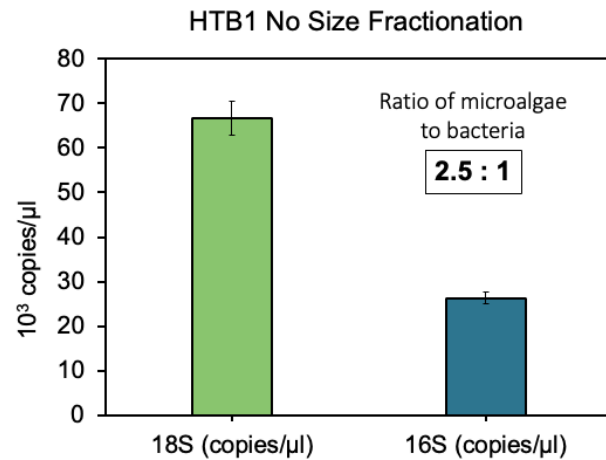


Figure 2.15 Digital PCR data displaying counts of *T. obliquus* HTB1 18S rRNA genes (green) and bacterial 16S rRNA genes (blue) with the microalgae to bacteria ratio being 2.5:1.

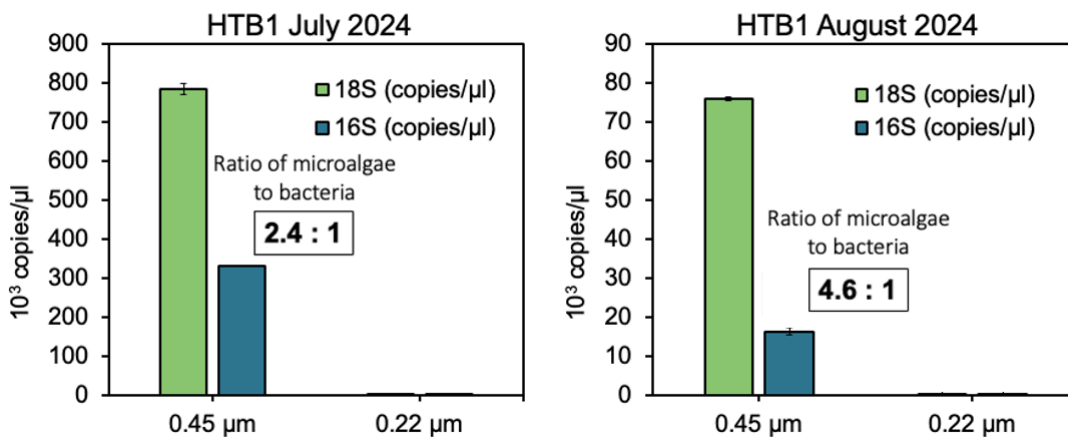


Figure 2.16 Digital PCR data displaying counts of *T. obliquus* HTB1 18S rRNA genes (green) and bacterial 16S rRNA genes (blue) after cultures were filtered onto 0.45 μm filters with flow-through then being filtered on 0.22 μm .

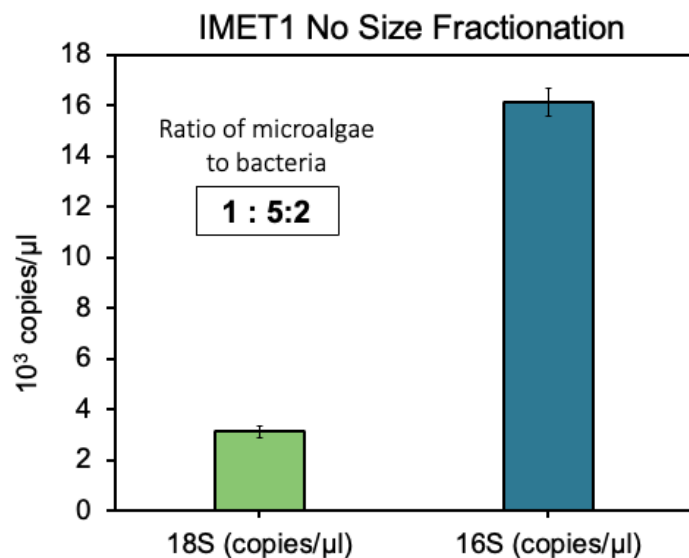


Figure 2.17 Digital PCR data displaying counts of *N. oceanica* IMET1 18S rRNA genes (green) and bacterial 16S rRNA genes (blue) with the microalgae to bacteria ratio being approx. 1: 5.2.

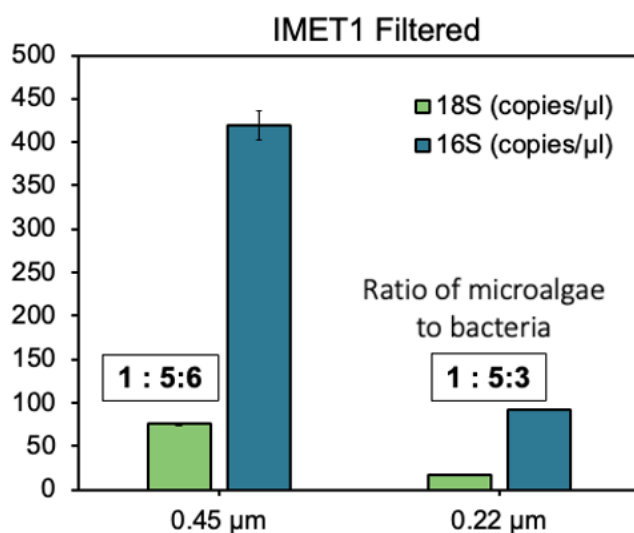


Figure 2.18 Digital PCR data displaying counts of *N. oceanica* IMET1 18S rRNA genes (green) and bacterial 16S rRNA genes (blue) after cultures were filtered onto 0.45 μm filters with flow-through then being filtered on 0.22 μm; ratio remains approx. 1: 5.2.

2.5 Discussion

2.5.1 A distinction can be made between closely associated bacteria and free-living symbionts in microalgal cultures

In this study, closely associated symbionts were defined as the bacteria retained on a 0.45 μm filter along with microalgal cells. This fraction includes the endosymbionts within microalgal cells and those bacteria loosely or closely attached to the microalgal cell surface. Other studies have fractionated bacteria in microalgal cultures using similar methods albeit with various filter sizes, e.g. 5 μm (Rooney-Varga *et al.*, 2005), 3 μm (Sapp *et al.*, 2007; Jacob *et al.*, 2024) and 1 μm (Riemann *et al.*, 2000). Considering the small size of *N. oceanica* IMET1 at *ca.* 2 μm (Figure 2.9), 0.45 μm pore size was deemed an appropriate filter size that would retain microalgae and associated bacteria but allow free-living bacteria to pass through in the filtrate. Phylogenetic and functional differences between free-living and closely associated bacterial populations have been identified in several microalgal species. This is true both in natural algal blooms (Riemann *et al.*, 2000; Fandino *et al.*, 2001) and in laboratory settings (Grossart *et al.*, 2005; Jacob *et al.*, 2024). Sapp *et al.* (2007) showed that bacteria closely associated with a diatom culture mainly belonged to the Bacteroidetes phylum (namely, the *Flavobacteria–Sphingobacteria* group) and that the free-living fractions comprised of Alphaproteobacteria (Roseobacter group). This corroborates a previous finding in a dinoflagellate bloom where closely associated bacteria belonged to the *Flavobacteria–Sphingobacteria* group within Bacteroidetes with α - and γ -*Proteobacteria* making up the free-living fraction (Fandino *et al.*, 2001). In the same study, bacteria within the surrounding water were phylogenetically distinct from those associated with the bloom. While many studies focus on diatoms and dinoflagellates (Riemann *et al.*, 2000; Grossart *et al.*, 2005; Jasti *et al.*, 2005; Rooney-Varga *et al.*, 2005), differences in free-living and

attached bacterial populations have also been previously observed in *Nannochloropsis* sp. (Samo *et al.*, 2018). Other studies have revealed phylogenetic differences among free-living and particle-attached/aggregated bacteria outside of algal systems (DeLong *et al.*, 1993; Bidle and Fletcher, 1995; Acinas *et al.*, 1999). In the study on spatial associations described in this chapter, there were no marked morphological differences observed between associated and free-living bacterial communities associated with either microalgal species. However, the detailed community analysis described in Chapter 3 provides more detailed insights into differences between associated and free-living bacterial communities.

2.5.2 Bacterial attachment and contributions to the phycosphere

Bacterial colonization of microalgae has been observed in nature for decades (Kogure *et al.*, 1981; Paerl, 1996). Colonization often depends on the stage of algal blooms with attachment coinciding with later phases (Veldhuis *et al.*, 1986; Smith *et al.*, 1995; Riemann *et al.*, 2000). Research focused on the microalgal phycosphere has elucidated the mechanisms behind bacterial recruitment and attachment. Algal cells are known to release a large proportion of their total fixed carbon into their surrounding cell surface in the form of extracellular polymeric substances (EPS), with exopolysaccharide² accounting up to 90% of the total polymers (Myklestad, 1995; Naveed *et al.*, 2019). Exopolysaccharides are the primary molecules driving recruitment and aggregation, creating a physical space in which bacterial cells can adhere (Smriga *et al.*, 2016; Xiao and Zheng, 2016). Xiao and Zheng (2016) list the various combinations of monosaccharides that are present in the microalgal EPS including glucose, galactose, fucose,

² Extracellular polymeric substances and exopolysaccharides are both abbreviated EPS, and the two terms are often used interchangeably.

xylose, arabinose, rhamnose, mannose, fructose, and/or sugar derivatives. The proportion of sugars, as well as extracellular proteins and amino acid content (also reviewed by Xiao and Zheng, 2016), is dependent on taxonomy and environmental conditions (Mühlenbruch *et al.*, 2018). These variations in the EPS pool could be a driving factor for the species specificity seen in bacterial colonization. While the majority of algal exudates are rapidly consumed and remineralized by the bacterial community (Møller Jensen, 1983; Bratbak and Thingstad, 1985; Obernosterer and Herndl, 1995), evidence has been found that bacteria also contribute matter to the polysaccharidic matrix of the phycosphere. Lipsman *et al.* (2024) found a biosynthetic pathway responsible for a bacterial-derived exopolysaccharide found in the phycosphere with increased expression observed in algal-rich environments. It is clear that algal exudates play a key role in promoting colonization of bacteria who contribute exopolysaccharides in return. This milieu of compounds may contribute to enhanced microalgal growth. *Phaeodactylum tricornutum* showed improved growth in the presence of bacterial spent medium, possibly due to the presence of bacterial exudates (Daly *et al.*, 2023) and growth enhancement from such exudates has been previously reported (Bruckner *et al.*, 2011). The several studies reporting growth enhancement and species-specific interactions between algae and their symbiont fractions lead to the conclusion that spatial associations are key in understanding culture dynamics. Gaining a better understanding of these dynamics will be helpful in engineering algal systems with denser biomass and desirable cell content.

2.5.3 Microscopy as a tool to visualize microalgal-bacterial consortia

Both *N. oceanica* IMET1 and *T. obliquus* HTB1 have been visualized by light microscopy previously and published micrographs are consistent with those of this study (Suda

et al., 2002; do Carmo Cesário *et al.*, 2022). Fawley and Fawley (2007) interestingly showed that different freshwater *Nannochloropsis* species can be distinguished from one another by light microscopy. However, marine *Nannochloropsis* species typically require molecular analysis for reliable identification (Fawley and Fawley, 2007). Epifluorescent microscopy can reveal microalgae and bacteria as distinct cells without relying on high magnification. This is in part because microalgae contain chlorophylls as autofluoresce pigments (Takahashi, 2019). Autofluorescence of microalgal pigment can provide clear evidence on size, shape, and quantity of microalgal cells (Figure 2.4). However, the signal intensity of autofluorescence can be a major impediment to distinguishing algal cells from bacterial cells. The remarkable efficiency of chlorophyll lies in its ability to use a broad spectrum of light to drive the photosynthetic process. As such, the chlorophyll pigment can be excited by the majority of the visible light spectrum (Billinton and Knight, 2001). The spectra of chlorophyll and other algal pigments often overlap with the emission wavelengths of many fluorescent dyes, thus erasing bacteria from the field of vision (Dashkova *et al.*, 2016). This spectral overlap is often a problem in microscopy and flow cytometry (Hyka *et al.*, 2013; Carreira *et al.*, 2015). The approach of this study was to use a fluorescent dye with minimal overlap to chlorophyll. Table 2.2 shows that chlorophyll generally excites at 430-450 and 640-660 nm while giving off a fluorescent signal of 640-680 nm. I first chose SYBR Gold and SYBR Green (495 nm excitation and ~530 nm emission). While the spectra did not overlap, both dyes had a high permeability rate across the membrane of IMET1 and HTB1. Issues with the membrane permeability of fluorescent dyes have been previously reported (Thornton, 2014). DAPI was then chosen as it is well-documented as a membrane-impermeable nucleic acid stain (Yoshinari *et al.*, 2024). A multilaser confocal microscope was

then used to separate emission signals and visualize microalgae and bacteria in one image (Figures 2.7 and 2.8).

To deal with obstacles for visualizing microalgae and bacteria using epifluorescent microscopy, other methods such as SEM can serve as helpful supplementations to elucidate spatial associations. The SEM micrographs of *T. obliquus* produced by do Carmo Cesário *et al.* (2022) were nearly identical to that of this dissertation, although with slight differences in morphology. The *T. obliquus* visualized by do Carmo Cesário had a slimmer “banana-shaped” morphology while Figures 2.10 and 2.13 show a wider “lemon-shaped” cell. The same study used TEM to visualize the cell wall and organelles of *T. obliquus*. *N. oceanica* has also been visualized previously with SEM (Cheng *et al.*, 2022; Savvidou *et al.*, 2022). While several studies have visualized microalgae and bacterial symbionts using SEM (Krohn-Molt *et al.*, 2013, 2017; Schmeisser *et al.*, 2017, among others), none have focused on the two species of this work. All preparation for microscopy depends on the manipulation of samples to wash, fix, dehydrate, and mount cells. Pipetting and centrifugation can disrupt some of the physical associations between microalgae and bacteria. Although close spatial associations can still be seen (Figures 2.11 and 2.13), there are likely more bacteria on the cell surface that were disrupted during sample preparation.

2.5.4 Quantification of microalgae and bacteria

To determine ratios of microalgal and bacterial cells in nature or within a laboratory consortium, it has been common practice to stain cells and count through microscopy or flow cytometry (Marie *et al.*, 1997; Riemann *et al.*, 2000; Wang *et al.*, 2010). Newer methods including dPCR quantification are becoming more common especially due to the ability to

quantify absolute abundances for low copy number targets (Doi *et al.*, 2015). Several dPCR approaches have been developed including microfluidic chip-based dPCR (Madic *et al.*, 2016), digital droplet PCR (Hindson *et al.*, 2011), and nanoplate-based dPCR systems (this study). Using nanoplate dPCR, microalgae and bacteria enriched from anaerobic digestion effluent were quantified by amplifying the 18S rRNA gene and a bacterial-specific 16S rRNA gene, respectively (Yu *et al.*, 2022; H. Yu *et al.*, 2023). To my knowledge, the two papers by Yu *et al.* and this dissertation are the only studies to use dPCR to quantify microalgae and bacteria. Although the method was first developed decades ago (Vogelstein and Kinzler, 1999) and has been independently developed several times, dPCR has just recently regained popularity in use (Morley, 2014). The Minimum Information for Publication of Digital PCR Experiments (dMIQE) guidelines were published to set standards within the methodology and reflect the growing use of dPCR (Whale *et al.*, 2020). In accordance with these guidelines, samples in this study were not multiplexed, templates were amplified at various ten-fold dilutions, primer and probe concentrations were set as per manufacturer's instructions, and automatic thresholds as well as Poisson statistics were used during analysis (Whale *et al.*, 2020). The ratio of five bacterial cells to each IMET1 cell is in the range typically found in studies of bacterial abundance in microalgal cultures. It was somewhat surprising that the number of bacteria associated with HTB1 was less than one bacterial cell for every two HTB1 cells, but this count was found in multiple cultures by two independent approaches: dPCR and microscopic estimates. Although this number of bacterial symbionts is low, the molecular community analysis described in Chapter 3 showed a consistent and stable bacterial community associated with HTB1, supporting a significant role for these bacteria even though their numbers are relatively low.

Chapter 3: Exploring the bacterial communities associated with microalgae through analysis of the 16S rRNA gene

3.1 Abstract

Symbiotic associations between microalgae and bacteria have become a focus in microalgal biotechnology due to the various advantages offered to both organisms, including enhancement of microalgal growth. In this chapter, I characterized the prokaryotic communities associated with laboratory-maintained 1 L cultures of *Tetradismus obliquus* strain HTB1 and *Nannochloropsis oceanica* strain IMET1 bubbled with air and 10% CO₂. I then characterized prokaryotic communities in both microalgal cultures bubbled with 5% CO₂ in large-scale 500 L and 6,800 L photobioreactors. In all analyses, the bacterial communities have been methodologically separated into two categories: “closely associated” symbionts, including attached symbionts and endosymbionts, are retained with the microalgae on filtration through a 0.45 µm filter. Subsequently, “free-living” symbionts that pass through the 0.45 µm filter are collected on a 0.22 µm filter. The dominant members of the *T. obliquus* bacterial communities were: *Algoriphagus aquaeductus*, *Erythrobacter* sp., and *Allorhizobium* sp. The dominant members of the *N. oceanica* bacterial communities were: *Gracilimonas sediminicola*, *Microcella pacifica*, and *Maricaulis maris*. *Tetradismus* and *Nannochloropsis* cultures shared two symbionts, both of which I was only able to classify to the phylum level (Patescibacteria) based on the 16S rRNA gene analysis described in this chapter. These two Patescibacteria dominated *N. oceanica* cultures bubbled with air and 10% CO₂ at the 1 L scale as well as both *N. oceanica* and *T. obliquus* bubbled with 5% CO₂ at the 500 L scale. The dominance of two Patescibacteria associated with both microalgae was striking and unexpected. This key finding was the driving factor for pursuing the recovery of metagenomic assembled genomes through long-read sequencing discussed in Chapters 4 and 5.

3.2 Introduction

3.2.1 Microalgal-bacterial consortia and mutualistic nutrient exchange

Pure culture cultivation of microalgae has been a focus in algal biotechnology, particularly for high-value products such as nutraceuticals. However, pure algal cultures are vulnerable to contamination by bacteria and are nearly impossible to keep axenic (Vu *et al.*, 2018). Although for some applications it may be necessary to keep cultures pure, such as single cell amplified genome sequencing, it is not generally economically viable to grow axenic algae cultures for large-scale production of high-value products. Further, bacterial “contaminants” often contribute positively to their algal hosts (Figure 3.1). For example, bacterial communities can enhance microalgal growth by excreting phytohormones and vitamins (González-González and De-Bashan, 2021; Lin *et al.*, 2022).

The interactions between microalgae and bacteria have been extensively studied in natural ecosystems. There are several ways in which bacteria and microalgae benefit from each other in natural ecosystems. One benefit both partners may receive is nutrient exchange. In aquatic systems, heterotrophic bacteria can obtain a large fraction of their carbon demand (up to 50%) directly from nearby algae (Fuhrman and Azam, 1982). Algae release highly labile, dissolved organic carbon (DOC) into the water that can then be utilized by neighboring bacteria (Figure 3.1). Heterotrophic bacteria can benefit greatly from these sources of larger polysaccharides and in return can provide algae with remineralized nutrients that are otherwise limiting (Figure 3.1). Another major contribution of bacteria to algae is vitamin B12, which is required for the synthesis of methionine. Many marine algae have been found to be rich in vitamin B12, but paradoxically more than 53% of the algal kingdom (171 out of the 326 algal species examined) were found to be B12-auxotrophs (Croft *et al.*, 2005). This indicates that for

more than half of algae, vitamin B12 is likely sourced from nearby bacteria. Croft et al. (2015) established that *Halomonas* sp., a bacterium known to produce B12, was able to support growth of two microalgae, *Amphidinium operculatum* and *Porphyridium purpureum*, to the same extent as exogenous vitamin B12. Not only do the microalgae benefit from bacterially-derived B12, but *Halomonas* sp. was able to survive within carbon-free media thus implying it used photosynthetically-derived DOC to grow (Croft *et al.*, 2005). Another major contribution of bacterial symbionts to microalgae in the natural environment are siderophores, especially when the siderophore undergoes photolysis nearby or within the phycosphere. One study that focused on photolysis suggests that bacteria promote algal assimilation of iron by facilitating photochemical redox cycling of bacterially derived Fe(III)-siderophore complexes (Amina et al., 2009).

Bacteria also have been found to supply microalgae with dissolved inorganic nitrogen (DIN), especially ammonia (NH₃) derived from nitrogen fixation. Considering many microalgae live in oligotrophic waters, their close associations with cyanobacteria could be driven by the fact that they can acquire N from the diazotrophy of their symbionts. For example, one study estimates that cyanobacteria of the genus *Richelia* can fix 81–744% more N than is needed for their own metabolism and that up to 98% of its fixed N is transferred to its diatom symbionts (Foster et al., 2011). Similarly, cyanobacteria may also provide microalgal partners with dissolved inorganic phosphate (DIP). Cyanobacteria—especially within the genus *Synechococcus*—can sequester phosphorus within a high-energy long-chained storage compound known as polyphosphate. Microalgae with attached or endosymbiotic cyanobacterial partners could potentially use this phosphorus in exchange for other dissolved nutrients, mostly DIC or

DOC. Much of the research on natural microalgae-bacteria interactions points to the likelihood that bacterial contributions may maintain microalgal metabolic performance.

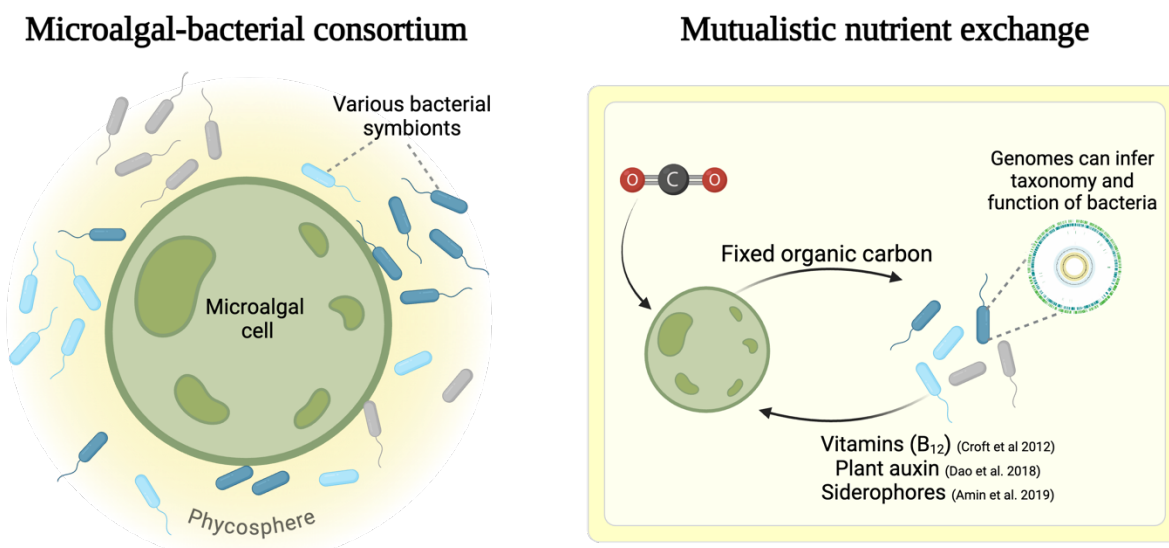


Figure 3.1 Conceptual diagram representing the microalgal phycosphere with various bacterial symbionts (left) and the nutrient exchange between both parties (right). Only some examples of bacterial input have been listed. Created in <https://BioRender.com>

3.2.2 Antagonistic antics between microalgae and bacteria

While much is known about the mutualistic exchanges between bacteria and microalgae, less attention has been paid to the antagonistic “tactics” used by one organism to control the physiology of the other. For example, certain bacteria are algicidal, meaning they excrete compounds that inhibit growth of or kill microalgae. The algal lysis leads to a spike in nearby nutrients that bacteria can scavenge (Ramanan *et al.*, 2016). Microalgae also have evolved strategies to control their microbiome populations and “weed out” potentially harmful residents. For example, microalgae produce and secrete antimicrobial peptides (AMPs) along with other

bioactive compounds with antimicrobial properties. These AMPs are a defense mechanism of microalgae, helping them combat bacterial, fungal, and viral pathogens. For example, the microalga *Chlorella sorokiniana* produces pepsin, a peptide that causes cell wall degradation of *Escherichia coli* (Ayswaria *et al.*, 2023). Microalgal-derived AMPs have gained attention for their pharmaceutical potential against human pathogens with resistance to traditional antibiotics. For example, peptides derived from the algae *Tetraselmis suecica* were active against pathogens including *Salmonella typhimurium* (causative agent of Salmonellosis) and methicillin-resistant *Staphylococcus aureus* (MRSA) (Ayswaria *et al.*, 2023). How microalgae interact with their naturally occurring bacterial symbionts remains unknown, particularly in their ability to selectively maintain beneficial bacteria while killing harmful ones.

Bacteria also secrete compounds that are antagonistic against their algal host. Quorum sensing (QS) is a dynamic process in which bacteria can sense the local cell density of their own species through a release of signaling compounds, most notably N-acyl-homoserine lactone (AHL) (Fuqua *et al.*, 2001). In addition to being transient signals that mediate coordinated gene expression among bacteria, QS molecules are often algicidal. Microalgae and bacteria have the ability to engage in cross-kingdom interactions through QS in response to one another's harmful compound release (Y. Zhang *et al.*, 2022). The presence of QS molecules has been shown to cause structural changes to the microalgal plastid as well as trigger differential expression of genes involved in photosynthesis (Dow, 2021).

3.2.3 Current research on industrially relevant microalgal-bacterial interactions

The symbiosis of bacteria and microalgae can provide many industrially relevant possibilities such as improving algal growth and lipid production. Within microalgal

biotechnology research, many microalgal cultures are maintained in co-culture with specific bacteria, thus creating an ‘artificial consortium’ with desired traits. There is evidence of direct enhancement of algae growth in co-culture with bacteria (Yao *et al.*, 2019; Palacios *et al.*, 2022). For example, bacteria can produce the phytohormone, IAA, that promotes microalgal growth; ten bacterial strains found to produce and secrete IAA were recently isolated from *Scenedesmus* sp. cultures (Dao *et al.*, 2018). *Azospirillum brasilense* has become a model growth-promoter for microalgae as it has been repeatedly found to secrete IAA and other organic compounds (Amavizca *et al.*, 2017; Peng *et al.*, 2020). For example, Peng *et al.*, 2021 found that *A. brasilense* promoted growth of two algal strains by 39–69%. Interestingly *E. coli* performed just as well as *A. brasilense* in this study and promoted growth by 16–64% (Peng *et al.*, 2021). This finding suggests that symbiotic enhancement of algal growth may be ubiquitous among many bacteria in the phycosphere. Bacteria have also been co-cultured with algae to enhance lipid production. For example, *E. coli* grown in association with the microalga, *Auxenochlorella protothecoides*, led to a doubling of the neutral lipid content (along with a two- to six-fold growth increase) (Higgins and VanderGheynsta 2014).

There are many challenges related to scaling up of microalgal cultures for large-scale production. These challenges include contamination of the microalgal cultures by undesirable bacteria, zooplankton, harmful microalgae, and viruses. Also, excess bacterial growth can lead to lower value products because of the contribution of bacterial biomass to the material harvested (Novoveská *et al.*, 2023). Desirable communities of associated bacteria, in addition to potentially promoting microalgal growth, can also play a role in reducing contamination by undesirable microbes.

Experimental methods to capitalize on the beneficial effects of bacteria generally involve co-culturing and sequencing methods. Prokaryotic community sequencing through amplification of the 16S rRNA gene (as well as metagenomic sequencing) can provide important insights for all algal biotechnology projects moving forward. To produce high density algal cultures with high lipid content, research must pay equal attention to algae and their bacterial symbionts.

3.2.4 Overview of this chapter

This chapter aimed to uncover the bacterial communities associated with *Nannochloropsis oceanica* IMET1 and *Tetradesmus obliquus* HTB1. As described in Chapter 2, the bacteria were separated into two categories: “closely associated” symbionts, including attached symbionts and endosymbionts, were retained on a 0.45 µm filter. Subsequently, “free-living” symbionts pass through the 0.45 µm filter and are collected on a 0.22 µm filter. Here I present bacterial communities from seven experiments in which the algae were grown under various CO₂ concentrations (0, 5, and 10%) and three different scales (1 L, 500 L, and 6,800 L). *N. oceanica* IMET1 was grown at the 1 L scale in the laboratory of Dr. Yantao Li. *T. obliquus* HTB1 was grown at the 1 L scale in the laboratory of Dr. Feng Chen. *N. oceanica* and *T. obliquus* were also grown at the 500 L and 6,800 L scales at HyTek Bio LLC, a local biotechnology company and collaborator. Filtration, DNA extractions, ethanol precipitations, and DNA verification through PCR and gel electrophoresis were conducted by me over the course of three years as a part of the Hill laboratory contribution to the U.S. Department of Energy project DE-FE0031914. To analyze sequence data, I used a custom bioinformatic pipeline, part of which I learned at the CBW-IMPACTT Microbiome Analysis Workshop in Calgary Canada in July of 2023. Results of this chapter show both microalgae have relatively

simple microbiomes that are distinct from one another. Microbiomes associated with both microalgae showed some similarities in multiple microalgal cultures grown in the laboratory and in large-scale photobioreactors, including the presence of cryptic and little-studied bacteria in the Patescibacteria phylum. The microalgae repeatedly shared two surprising symbionts, both belonging to the phylum Patescibacteria.

3.3 Methods

3.3.1 Growth experiments and prokaryotic community analysis of *T. obliquus* HTB1

Triplicate cultures of HTB1 were grown by Lauren Wanger in the laboratory of Dr. Feng Chen at IMET. A seed culture (70 ml) was inoculated in 630 ml BG11 medium, containing nitrate as the N source and with 5 mM Ca^{2+} and 0.01 M NaHCO_3 . Cultures were maintained at room temperature and exposed to $60 \mu\text{E m}^{-2} \text{s}^{-2}$ light. Cultures were bubbled with either ambient air or air enriched with 10% CO_2 for 14 days. On Day 12, the air enriched with 10% CO_2 was shut off and these cultures were bubbled with ambient air until the experiment concluded on Day 14. This was done to increase the pH within the system to induce formation of calcium carbonate (CaCO_3). Microalgal growth was measured through optical density, cell counting with a hemocytometer, dry weight, and ash-free dry weight. Samples of microalgal cultures were sent to me in the Hill laboratory on Day 0, 5, 10, and 14 for microbiome analysis.

For microbiome analysis, 20 ml of each microalgal culture was vacuum-filtered through a $0.45 \mu\text{m}$ cellulose acetate filter (Advantec) with a Nalgene Reusable Filter Unit (Thermo Scientific), and the filtrate was collected. The filtrate was subsequently filtered through a $0.22 \mu\text{m}$ Sterivex filter (Millipore Sigma) using sterile tubing and a peristaltic pump. DNA from cells retained on the $0.45 \mu\text{m}$ filters was extracted using the DNeasy Powerwater kit (Qiagen). DNA from cells retained on the $0.22 \mu\text{m}$ filters was extracted using DNeasy Powerwater Sterivex kit (Qiagen). DNA concentrations were checked by absorbance at 260 nm using a UV–visible spectrophotometer (NanoDrop, Thermo Scientific). To verify that bacterial DNA was successfully extracted, PCR was conducted using the universal 16S rRNA gene primer set 27F/1492R (Lane, 1991; Stackebrandt and Liesack, 1993). Reactions were carried out in 25- μl total volume including 12.5 μl JumpStart REDTaq ReadyMix (Sigma Aldrich), 1 μl of each

primer (initial concentration 10 μ M), approx. 30 ng of DNA template, and PCR-grade water. The PCR program consisted of 30 cycles of 95°C for 1 minute and 30 seconds, 55°C for 1 minute and 30 seconds, and 72°C for 1 minute and 30 seconds, with a final extension at 72°C for 7 minutes. PCR products were confirmed through gel electrophoresis on 1.5% TBE gels stained with ethidium bromide (10 mg/ml) and visualized under UV light. Ethanol precipitations were performed on samples where DNA bands were not visible, and PCR and gel electrophoresis was performed again. This was often the case for the 0.22 μ m filters; ethanol precipitations were often successful in concentrating DNA to levels suitable for sequencing. DNA was sequenced on an Illumina paired-end platform (MiSeq 2 x 300) at the BioAnalytical Services Laboratory (BASLab) at the Institute of Marine and Environmental Technology (IMET).

Sequence reads were analyzed using the QIIME2 pipeline and trimmed based on a Phred-quality score of 15 (95% base calling accuracy). Sequence quality control and Feature Table Construction was completed with DADA2 (offered through an R plug-in with QIIME2). Taxonomy was assigned ($\geq 99\%$ similarity) using the Naive Bayes classifier trained from the newest Silva database (version 132) that had been applied to the full length 16S rRNA gene. Taxonomic identities were confirmed manually through the NCBI blastn program. Microalgal chloroplast sequence was filtered from datasets. A schematic of the custom bioinformatic pipeline used in this chapter can be found in Figure 3.2

3.3.2 Growth experiments and prokaryotic community analysis of *N. oceanica*

IMET1

Triplicate cultures of IMET1 were grown by Dr. Yi-Ying Lee of the Li laboratory at IMET. IMET1 cultures were grown in triplicate bubbled with air or air enriched with 10% CO₂.

Seed cultures (80 ml) were inoculated in 700 mL F/2 medium, containing 10 mM urea as the N source and either with addition of 0.02 M NaHCO₃ or with no NaHCO₃ added (control). Our previous research has shown that 0.02 M NaHCO₃ promoted algal growth (Lee *et al.*, 2024; Jonas *et al.*, 2025a). Cultures were maintained for 14 days. The CO₂ supplementation was shut off on Day 12 and cultures were then bubbled with ambient air until the experiment concluded on Day 14. Samples were collected for microbiome analysis on Days 0, 4, 8, 12 (before CO₂ cutoff), and 14 to be sent to me in the Hill Laboratory. Microalgal growth was measured by optical density, cell counting with a hemocytometer, dry weight, and ash-free dry weight. Methods for filtering, DNA extraction, DNA confirmation, and sequence analysis for IMET1 were identical to that of HTB1, as described above. DNA extractions from experiment ‘DOE10’ were done by Dr. Daniela Tizabi when I was abroad in Colombia (Table 3.1). The scale-up testing was conducted in a 500 L and 6,800 L photobioreactor at Hy-Tek Bio’s facility at the Baltimore Back River Wastewater Treatment Plant. In the case of filtering IMET1 seed cultures, some samples were filtered onto a 3 µm filter instead of 0.45 µm. This was in an attempt to filter out microalgal cells while retaining more bacteria. After passing 80 ml of microalgal culture through multiple 3 µm filters, the filtrate was collected and filtered through one 0.22 µm filter on which bacteria were retained.

3.3.3 Phylogenetic tree construction

Phylogenetic trees were created with Méthodes et algorithmes pour la bio informatique (MAB) (Dereeper *et al.*, 2008, 2010), alignment with MUSCLE (Edgar, 2004), a curation by Gblocks which eliminates poorly aligned positions and divergent regions (Castresana, 2000), PhyML for phylogeny (Guindon and Gascuel, 2003; Anisimova and Gascuel, 2006), and

TreeDyn for tree rendering (Chevenet *et al.*, 2006). For confidence analysis, data was resampled using 1,000 bootstrap replicates. TreeDyn and Adobe Illustrator were used for tree construction.

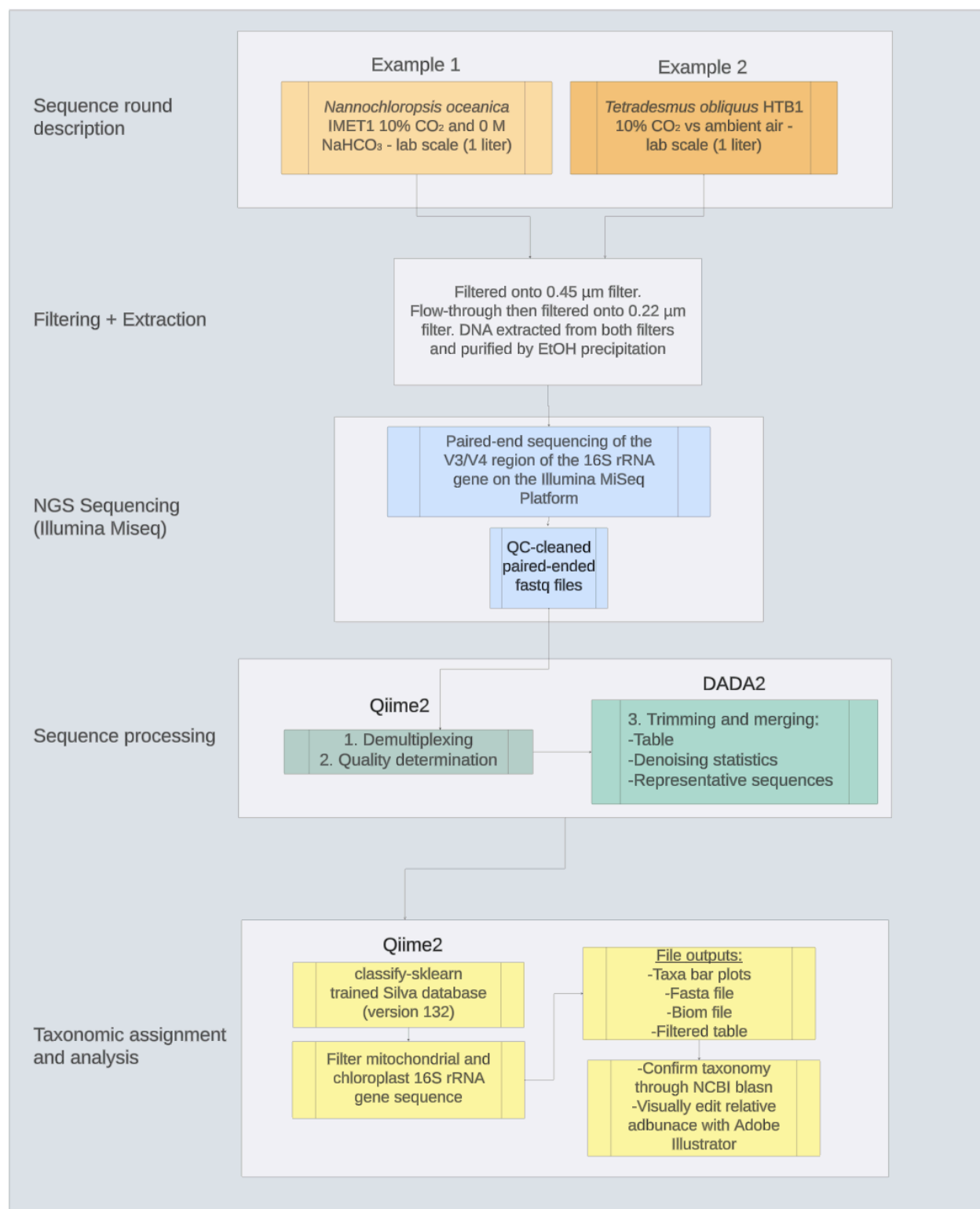


Figure 3.2 Pipeline depicting filtering protocols, DNA extraction methods, and bioinformatic programs used for microbiome data analysis.

3.4 Results

3.4.1 Bacterial communities associated with *Tetrademus obliquus* strain HTB1 at the 1 L scale

T. obliquus HTB1 was grown in the laboratory of Dr. Feng Chen and at Hy-Tek Bio in three separate experiments (Table 3.1). In October 2021, Lauren Wagner grew *T. obliquus* cultures at the 1 L scale with ambient air and 10% CO₂ ('DOE3' in Table 3.1). *T. obliquus* growth was enhanced under 10% CO₂ conditions (Figure 3.3). Day 0, 5, 10, and 14 were sent to the Hill laboratory for bacterial community analysis using 16S rRNA gene sequencing. The most relatively abundant bacterial taxon in these *T. obliquus* cultures was *Algoriphagus aquaeductus*, followed by *Erythrobacter* sp. and *Allorhizobium* sp. (Figure 3.4). A fourth bacterial species of significance is *Lacibacter* sp. (*cauensis*), which only appears in the cultures bubbled with 10% CO₂. High-quality metagenomes have been recovered for *Algoriphagus aquaeductus*, *Erythrobacter* sp. and *Allorhizobium* sp. and will be discussed in Chapter 4. A significant difference in beta diversity was found between the bacterial communities of the air and CO₂ treatments within the 0.45 µm fraction ($p = 0.002$) (Table 3.2). In addition, bacterial communities were significantly different between fractions in both the air and CO₂ treatments ($p = 0.003$ & 0.006 , respectively) (Table 3.2). The remaining two experiments with HTB1 at 500 L and 6,800 L will be discussed in Section 3.4.3.

Table 3.1 Details on seven experiments chosen for bacterial community analysis. Five experiments were with *N. oceanica* IMET1 and three were with *T. obliquus* HTB1. * = bioreactor failure. ‡ = results have been published.

Sequence Round Identifier	Date	Algae	Duration (days)	CO ₂ (%)	Laboratory	Other experimental conditions	Figure
DOE3 [‡]	December 2021	HTB1	14	0 10	Chen	Air vs. 10 % CO ₂	Figure 3.4
DOE6 [‡]	May 2022	IMET1	14	10	Li	0 M vs. 0.02 M NaHCO ₃	Figure 3.9
DOE10 [‡]	May 2023	IMET1	14	0	Li	0 M vs. 0.02 M NaHCO ₃	Figures 3.6 & 3.8
DOE 500 L [‡]	February 2023	Both	50	5	Hy-Tek Bio	500 L	Figure 3.10
DOE 6,800 L	December 2023	HTB1	n/a*	15	Hy-Tek Bio	6,800 L	Figure 3.11
DOE12	March 2024	IMET1	14	10	Li	0.02 M NaHCO ₃	Figure 3.12

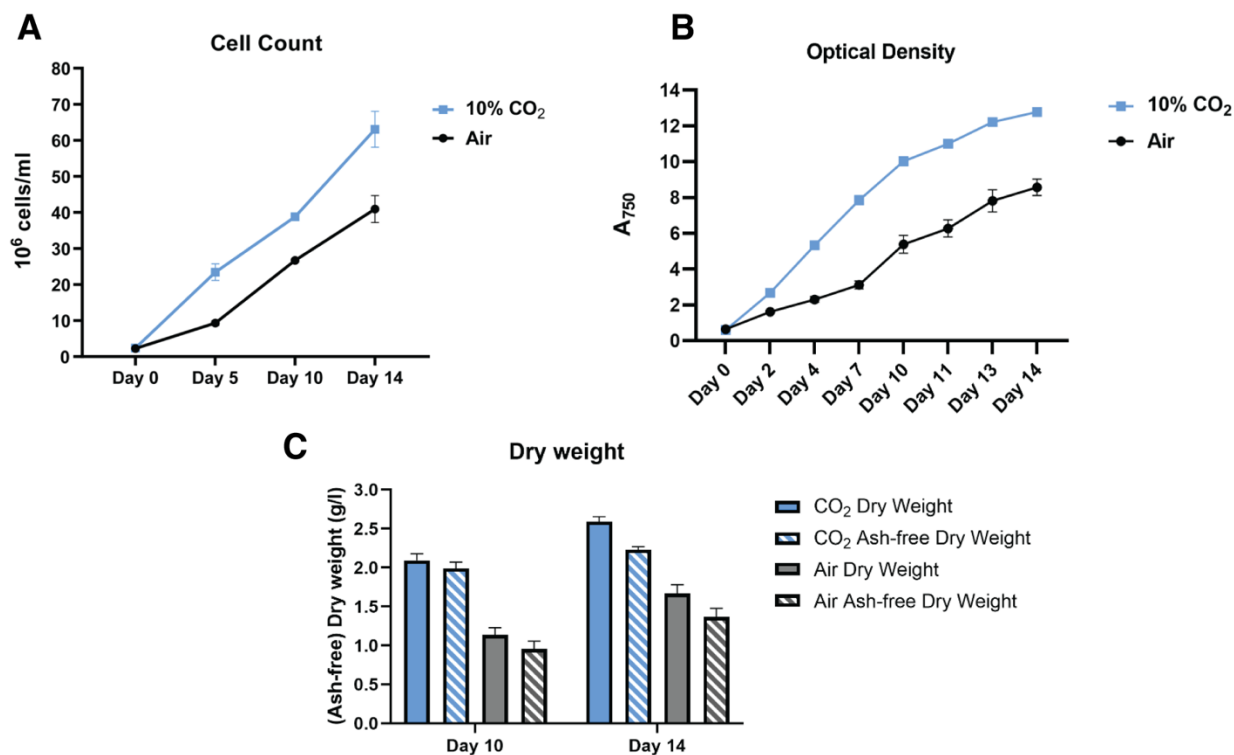


Figure 3.3 Growth data of *Tetradesmus obliquus* strain HTB1 grown with ambient air (black) and 10% CO₂ (blue) at the 1 L scale. (A) Cell count by hemocytometry, y axis in million cells/milliliter. (B) Optical density by spectrophotometry (Absorbance of 750 nm). (C) Dry weight (solid bars) and Ash-free Dry Weight measurements (striped) in grams/liter. Experiment performed by Lauren Wagner.

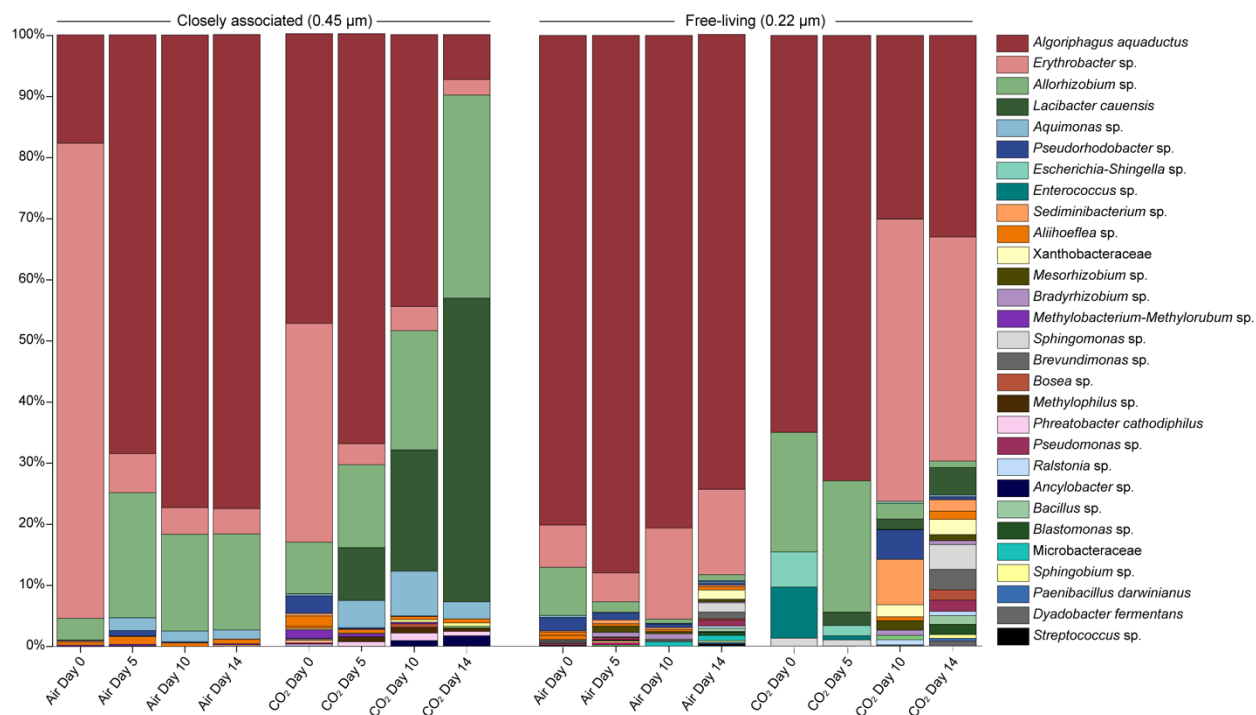


Figure 3.4 Relative abundance (analysis of the V3/V4 region of the 16S rRNA gene) of closely associated (left 10 panels) and free-living (right 10 panels) bacteria in cultures of *Tetradismus obliquus* HTB1 grown with ambient air and 10% CO₂. Cultures grown with 10% CO₂ were switched to air on Day 12. 16S rRNA gene sequences from *T. obliquus* chloroplasts have been removed bioinformatically. This experiment is labeled as DOE3 in Table 3.1.

Table 3.2 Pairwise t-test of beta diversity between bacterial communities found within *T. obliquus* cultures bubbled with air and 10% CO₂ at the 0.45 µm level, Air at the 0.22 µm and 0.45 µm level, and 10% CO₂ at the 0.22 µm and 0.45 µm level.

Group 1	Group 2	pseudo-F	p-value
Air – 0.45 µm	CO ₂ – 0.45 µm	7.16931339	0.002
Air – 0.45 µm	Air – 0.22 µm	4.53292109	0.003
CO ₂ – 0.45 µm	CO ₂ – 0.22 µm	8.54458792	0.006

3.4.1 *Nannochloropsis oceanica* IMET1 bubbled with 10% CO₂ or ambient air at the 1 L scale

N. oceanica IMET1 was grown by Dr. Yi-Ying Lee in the laboratory of Dr. Yantao Li and at Hy-Tek Bio in four separate experiments (Table 3.1). In the first experiment, *N. oceanica* was bubbled with ambient air for 14 days in 1 L bubbling columns and were given either 0 or 0.02 M NaHCO₃ (Figure 3.5 A-D). NaHCO₃ was added to the cultures to create high-pH and high-alkalinity conditions that would induce calcium carbonate (CaCO₃) precipitation (Jonas *et al.*, 2025a). In the second experiment, *N. oceanica* cultures were aerated with 10% CO₂ that simulated power plant flue gas for 12 days. The aeration was then switched to ambient air for two days (Figure 3.5 E-F). The switch to air was done to raise pH to favorable condition for CaCO₃ precipitation. The cultures bubbled with 10% CO₂ were also given either 0 or 0.02 M NaHCO₃. In each experiment, Days 0, 4, 8, 12, and 14 were sent to me in the Hill laboratory for bacterial community analysis using 16S rRNA gene sequencing.

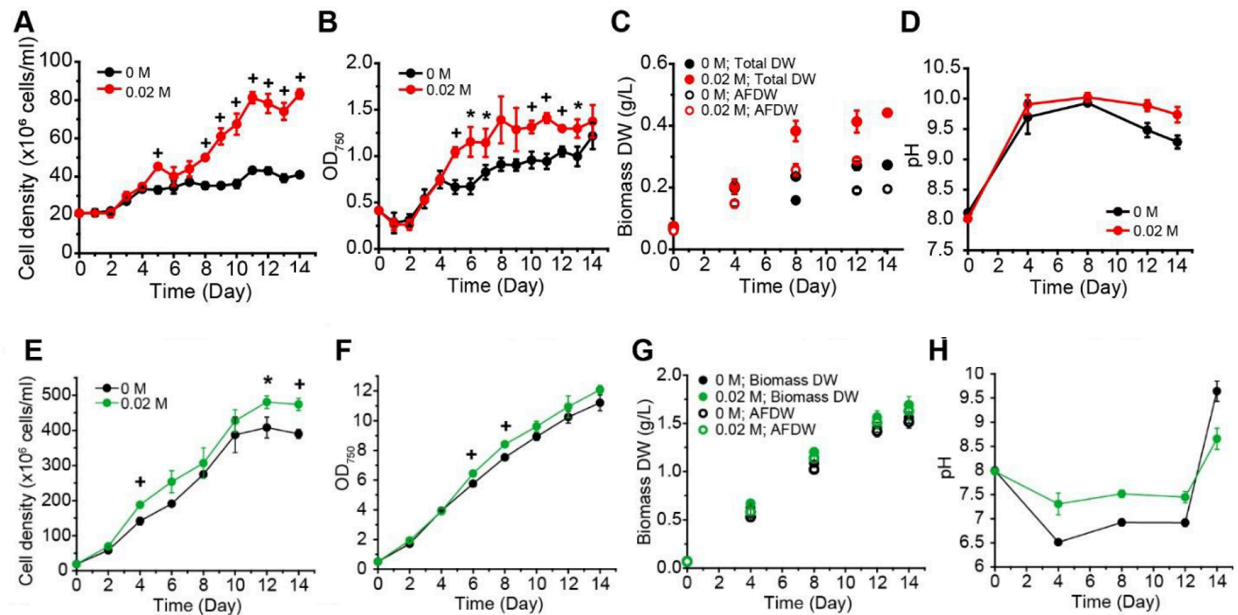


Figure 3.5 Performance of *Nannochloropsis oceanica* IMET1 cultures under ambient air (A-D, red lines) and 10% CO₂ (E-H, green lines) in the presence or absence of 0.02 M NaHCO₃ in 1 L column photobioreactors. The growth of the cultures was measured by cell density (A), optical density (B), biomass dry weight and ash-free dry weight (AFDW) (C). pH (D) is also shown. 10% CO₂ was switched to ambient air at Day 12, and the cultures continued to grow under ambient air until Day 14. Figure modified with permission from Jonas *et al.* (2025a).

3.4.2 Bacterial communities of *Nannochloropsis oceanica* IMET1 bubbled with air

The bacterial community of *N. oceanica* IMET1 bubbled with air was dominated by four species (Figure 3.6). In the bacterial community closely associated with *N. oceanica* strain IMET1, *Maricaulis maris* and *Gracilimonas* sp. were dominant on Day 0 and Day 4 regardless of whether microalgae were supplemented with NaHCO₃ or not. *Gracilimonas* sp. is now known to be *Gracilimonas sediminicola* (Figure 3.8). *Marinisubtilis pacificus* (now known to be *Microcella pacifica*) was also found albeit at a lower relative abundance. Interestingly, after

Days 0 and 4, all samples were completely dominated by a novel ‘uncultured bacterium clone’ (Figure 3.6) This discovery was published in early 2024 (Lee *et al.*, 2024) and was the basis for moving forward with metagenomic sequencing with the aim of recovering a genome.

Preliminary phylogenetic analysis (Figure 3.7) suggested that this novel bacterium was related to a member of the uncultured phylum, Patescibacteria. We now know the ‘uncultured bacterium clone’ is indeed two bacteria and the published figure has been modified in this dissertation in light of this new information (Figure 3.8). High-quality metagenomic assembled genomes (MAGs) have been recovered for these two bacteria, as well as *Microcella pacifica* and *Gracilimonas sedminicola*. Through phylogenomic analysis of the uncultured bacterium clones, I have classified these two bacteria as *Phycocordibacter aenigmaticus* gen. nov. sp. nov. and *Minusculum obligatum* gen. nov. sp. nov. (Jonas *et al.*, 2025b [in review]). These bacteria along with the other recovered MAGs will be discussed in Chapter 4.

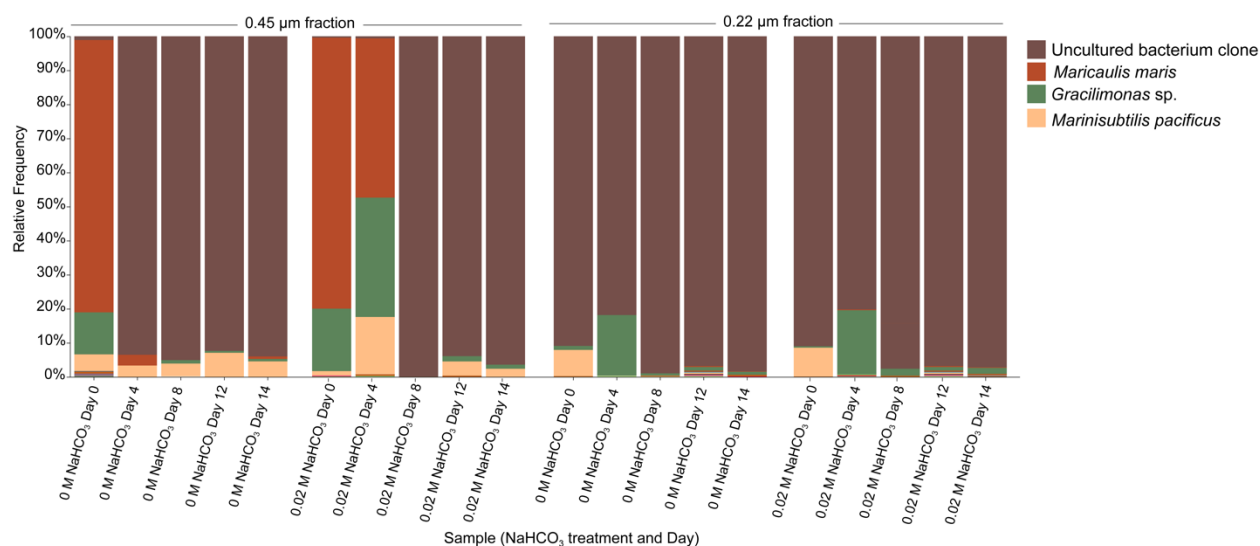


Figure 3.6 Original published analysis showing relative abundance of closely-associated prokaryotic community (0.45 µm fraction – first ten bars) and free-living prokaryotic community (0.22 µm fraction – remaining ten bars) of *N. oceanica* IMET1. Sequencing was targeted on the

V3/V4 hypervariable region of the 16S rRNA gene. 16S rRNA gene sequences derived from chloroplasts have been removed bioinformatically. Figure 3.7 has been modified and displayed with permission from (Lee *et al.*, 2024). This experiment is labeled as DOE10 in Table 3.1.

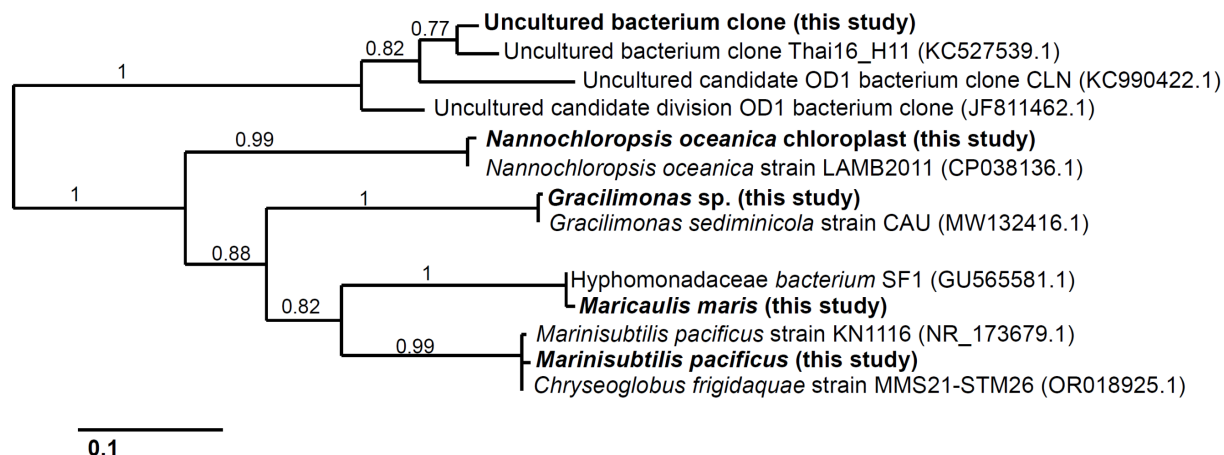


Figure 3.7 Phylogenetic tree of partial 16S rRNA gene sequences associated with *N. oceanica* IMET1 microalgal cultures bubbled with ambient air. Sequences from this study are in bold. Nucleotide alignment was conducted with MUSCLE and curation with a built-in Gblocks utility. The tree was constructed using the maximum likelihood method and boot strap confidence values (expressed as percentages of 1000 replications) are shown at branch nodes. Branches with bootstrap values less than 50% were eliminated. Figure displayed with permission from Lee *et al.* (2024).

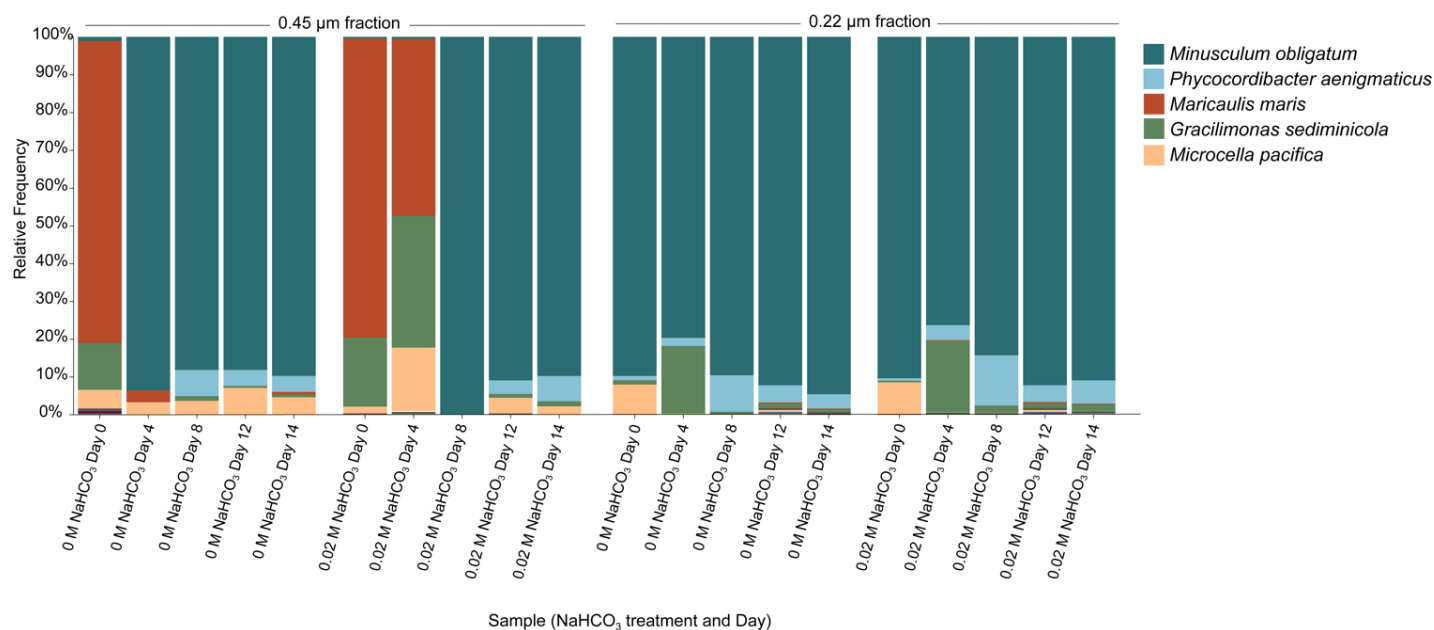


Figure 3.8 Modified relative abundance plot based on Figure 3.6, with brown bars in Figure 3.6 now shown as two distinct bacteria: *Minuscolum obligatum* (dark teal) and *Phycocordibacter aenigmaticus* (light blue). This experiment is labeled as DOE10 in Table 3.1.

3.4.3 Bacterial communities of *Nannochloropsis oceanica* IMET1 bubbled with 10% CO₂

The same two Patescibacteria, *Phycocordibacter aenigmaticus* and *Minuscolum obligatum* (labeled as ‘uncultured bacterium clone’ in Figures 3.6) were found in *N. oceanica* cultures bubbled with 10% CO₂ (Figure 3.9). *M. obligatum* was found to be a dominant symbiont found across all samples, regardless of NaHCO₃ input, day of experiment, or filter fraction size (Figure 3.9 - dark teal bars). Other dominant species included *Maricaulis maris*, *Balneola alkaliphila*, and *Microcella pacifica*. Other notable symbionts of IMET1, albeit at lower relative abundance, were *Gracilimonas sediminicola*, *Roseivirgia spongicola*, *Reichenbachiella* sp., *Marivirgia* sp., *P. aenigmaticus*, and an uncultured gammaproteobacterium. Lastly, *Leptonema*

sp. sequence was repeatedly detected in association with *N. oceanica* and was imaged by SEM (Figure 2.12 | Chapter 2). Alpha diversity was analyzed using Faith's Phylogenetic Diversity which incorporates phylogenetic relationships between the amplicon sequence variants (ASVs) and is unweighted. In general, there was no significant difference in alpha diversity between samples receiving no NaHCO_3 (0 M) and 0.02 M NaHCO_3 or between samples of different fraction sizes, 0.45 μm vs. 0.22 μm , comprising closely associated and free-living bacteria, respectively (Appendix, Figures A3.1 and A3.2). The only samples that showed a significant difference in alpha diversity were Day 12 samples within the 0.22 μm fraction compared to Day 0 and Day 8 (p-value = 0.04). (Appendix, Figure A3.3). Beta diversity was analyzed with Unifrac distances (unweighted). When comparing samples across timepoints (Day), regardless of size fractionation or addition of NaHCO_3 , samples from Day 12 had a significant difference in beta diversity when compared to Day 0, p-value= 0.04. (Appendix, Figure A3.4). There was a significant difference in beta diversity between the 0.45 μm and 0.22 μm fraction, p-value= 0.006 (Appendix, Figure A3.5). NaHCO_3 input alone had no effect on beta diversity but did cause significant differences in beta diversity within the 0.22 μm fraction, p-value= 0.022 (Appendix, Figures A3.6 and A3.7). There was only a significant difference (p-value = 0.013) between 0.45 μm -fractionated samples supplemented with 0 M NaHCO_3 and 0.22 μm -fractionated samples supplemented with 0.02 M NaHCO_3 which makes sense given the significant factors on beta diversity listed prior (Appendix, Figure A3.7).

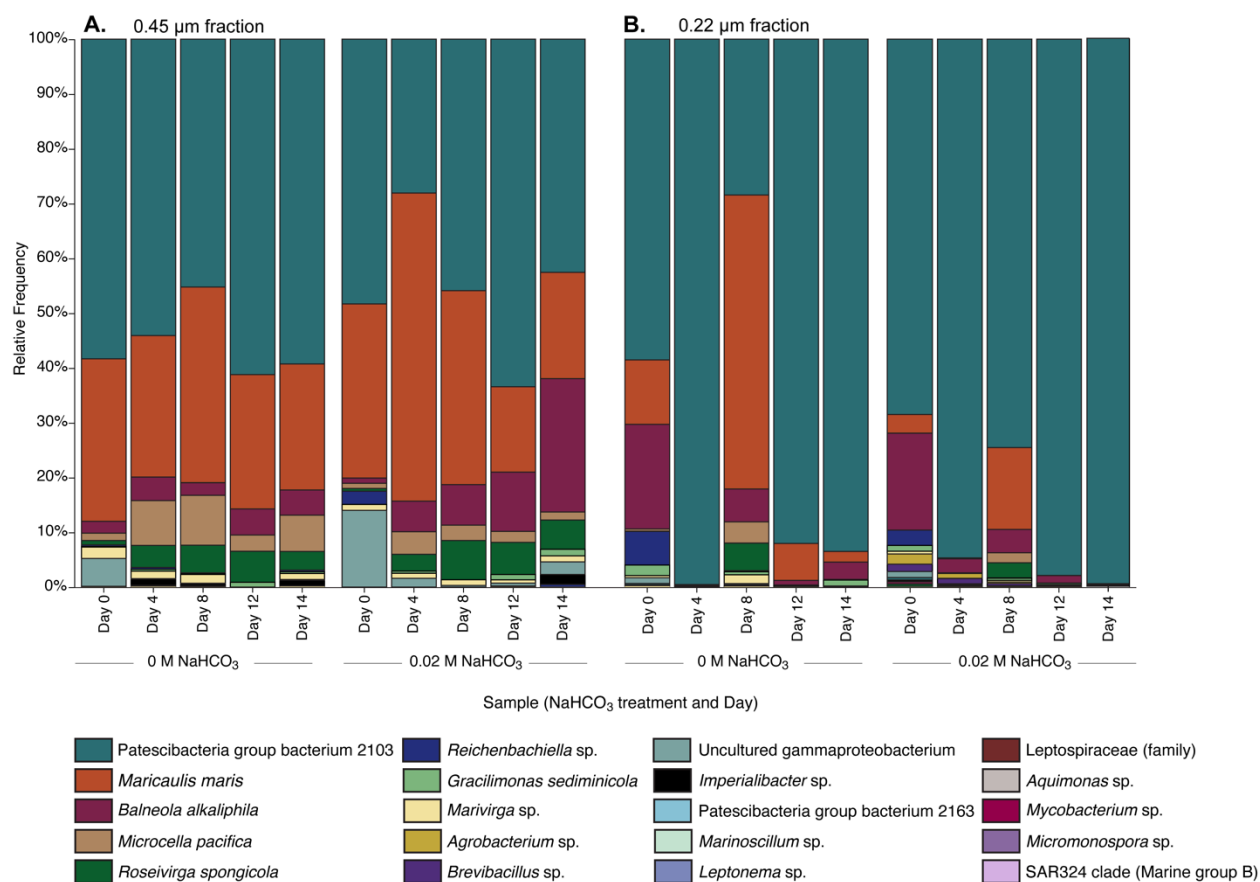


Figure 3.9 Relative abundance plots of the closely associated prokaryotic community (0.45 μm fraction) and free-living prokaryotic community (0.22 μm fraction) of *N. oceanica* IMET1 through 16S rRNA gene sequence analysis. IMET1 was grown at 1-liter scale with 10% CO_2 for 12 days, then grown with ambient air until Day 14. Cultures were supplemented with either no addition (0 M) or 0.02 M addition of NaHCO_3 . 16S rRNA gene sequences from *N. oceanica* chloroplasts were removed bioinformatically. Figure displayed with permission from Lee *et al.* (2024). This experiment is labeled as DOE6 in Table 3.1.

3.4.4 *N. oceanica* IMET1 and *T. obliquus* HTB1 bubbled with 5% CO₂ at the 500 L and 6,800 L scale

Scale-up testing was conducted in a 500 L and 6,800 L photobioreactor at Hy-Tek Bio's facility at the Baltimore Back River Wastewater Treatment Plant (BRWWTP). Both *N. oceanica* and *T. obliquus* were grown at the 500 L scale and were sparged with simulated boiler flue gas (5% CO₂) (Figure 3.10). Only *T. obliquus* was grown at the 6,800 L scale and was also bubbled with 5% CO₂. This system had a physical failure (burst bioreactor); one sample was taken from the bioreactor before the crash (Figure 3.11). There is no data available for *N. oceanica* grown at the 6,800 L scale as the bioreactor had to be replaced. Interestingly, at the 500 L scale in field bioreactors, the bacterial communities of *N. oceanica* and *T. obliquus* cultures remained similar to laboratory culture: the two aforementioned Patescibacteria, *P. aenigmaticus* and *M. obligatum*, were abundant, particularly in the 0.22 µm fraction (Figure 3.10). Within the 0.45 µm fraction of both microalgae, the bacterial communities have a high evenness. *Allorhizobium* sp., *Novospingobium* sp., *Reyranella* sp., *Candidatus Obscuribacter* sp., *Methylophilus* sp., and an uncultured member of the Bacterioidetes phylum were found within the *T. obliquus* HTB1 culture (Figure 3.10). *M. obligatum* and *P. aenigmaticus* are found within the *N. oceanica* microbiome at the 0.45 µm fraction along with *Parvibaculum* sp., *Gimesia* sp., *Tagaea* sp., and members of the Alphaproteobacteria and Gammaproteobacteria phyla and Rhodobacteriaceae family (Figure 3.10). At the 0.22 µm fraction, *M. obligatum* and *P. aenigmaticus* become dominant, making up >50% relative abundance in the *T. obliquus* cultures and >95% relative abundance in the *N. oceanica* cultures. One bacterium associated with *T. obliquus*, *Allorhizobium* sp., was found at both the 500 L and 1 L scale (Figure 3.4, Figure 3.10).

In the 6,800 L bioreactors, bacterial communities associated with *T. obliquus* were distinct from those of the 1 L and 500 L cultures (Figure 3.11). The community within the 0.45 μm fraction had a high evenness with ten species present: *Bacillus* sp., an uncultured member of the Rickettsiales order, an uncultured Grammaproteobacteria, uncultured members of the Gemmataceae and Vicinamibacteraceae families, *Sediminibacterium* sp., *Oligoflexus* sp., *Legionella* sp., *Bdellovibrio exovorus*, and an uncultured bacterium clone. The 0.22 μm fraction has a higher richness with the dominant members being *Bradyrhizobium* sp., *Bacillus* sp., *Massilia* sp., and *Acidovorax* sp. (Figure 3.11).

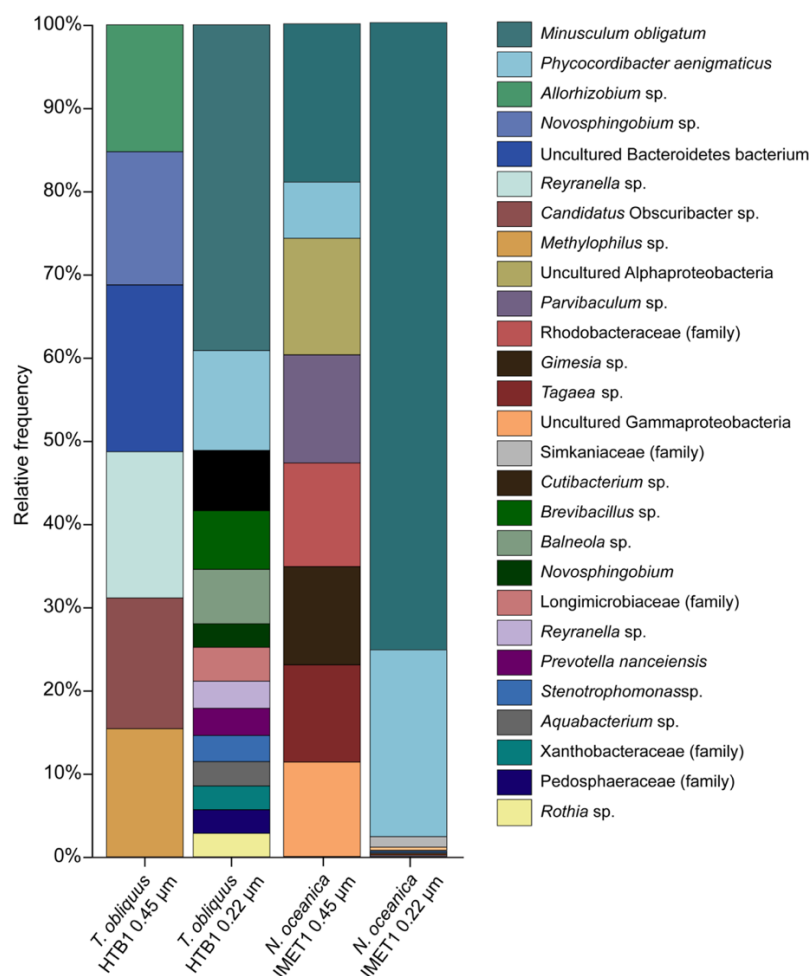


Figure 3.10 Relative abundance plots of closely associated prokaryotic community (0.45 µm fraction) and free-living prokaryotic community (0.22 µm fraction) of *T. obliquus* HTB1 and *N. oceanica* IMET1 through analysis of the V3/V4 region of the 16S rRNA gene. Both microalgae were grown at the 500-liter scale with air enriched with 5% CO₂. 16S rRNA gene sequences from chloroplasts have been removed bioinformatically. This experiment is labeled as DOE 500 L in Table 3.1.

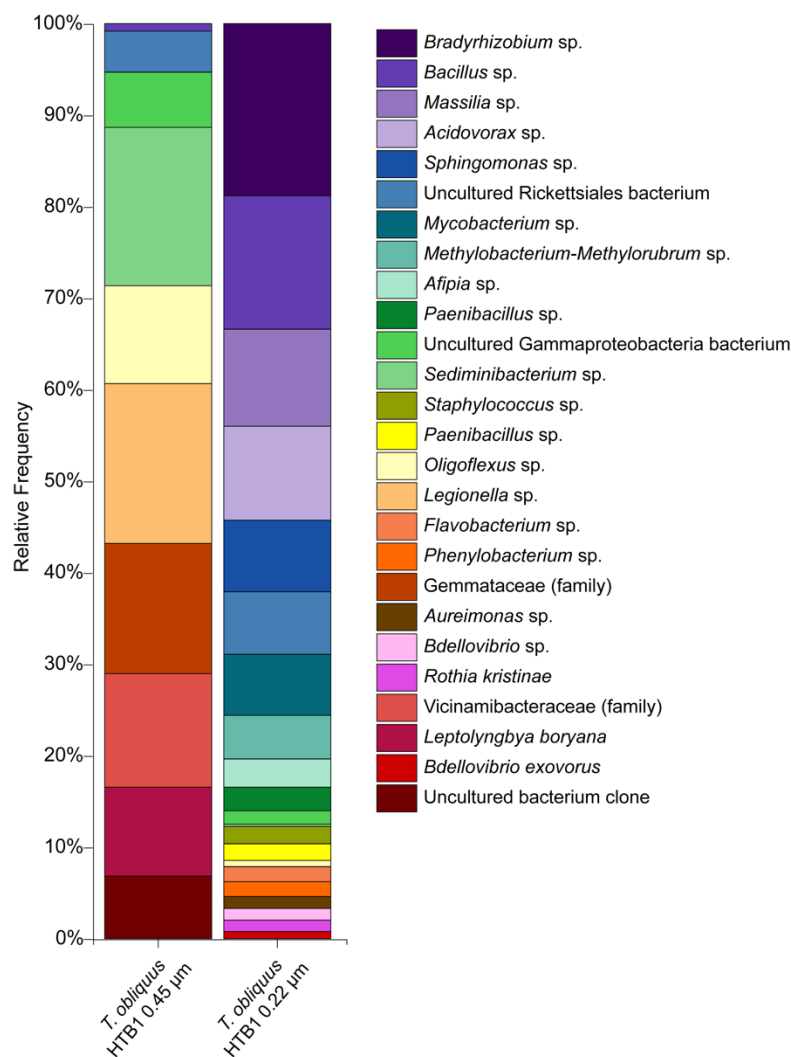


Figure 3.11 Relative abundance plots of closely associated prokaryotic community (0.45 µm fraction) and free-living prokaryotic community (0.22 µm fraction) of *T. obliquus* HTB1 through analysis of the V3/V4 region of the 16S rRNA gene. HTB1 was grown at the 6,800-liter scale with air enriched with 5% CO₂. 16S rRNA gene sequences from chloroplasts have been removed bioinformatically.

4.3.5 Bacterial communities vary within different *N. oceanica* seed cultures

At various time points through 2023 and 2024, bacterial community analysis was conducted on various *N. oceanica* seed cultures. This was done in an attempt to find more

samples with *M. obligatum* and *P. aenigmaticus* to send for metagenomic sequencing that was described in Chapter 4. Three seed cultures that started from three different *N. oceanica* colonies were subcultured and maintained in the laboratory by Kieran Szelong, Dr. Yi-Ying Lee, and me for future experiments (Table 3.3). The methods for filtering bacteria varied in each experiment and are described in the section 3.3.2. The bacterial communities of these seed cultures were markedly different from those of experiments described previously with *N. oceanica* at the 1 L and 500 L scale. The seed culture communities were also distinct from one another (Figure 3.12). The first four bars in Figure 3.12 show a seed culture maintained by me during November, 2023 that was filtered through both a 3 µm and a 0.45 µm filter before subsequent filtration with a 0.22 µm filter on which bacterial cells were retained. Dominant symbionts included a member of the Flavobacteriaceae family, *Marinobacter* sp., *Marinoscillum* sp., and *Ketobacter* sp. (Figure 3.12). The bacterial community from a seed culture maintained by Kieran Szelong also had the same member of the Flavobacteriaceae family and *Marinobacter* sp. with other distinct members such as *Magnetospira* sp. and *Arenibacter* sp. (Figure 3.12). The bacterial community from a seed culture maintained by Dr. Yi-Ying Lee was most similar to previous experiments and was dominated by *Magnetospira* sp., *B. alkaliphila*, a member of the Porticoccaceae family, *Bacillus* sp., *Muricauda* sp., and *Marinoscillum* sp. (Figure 3.12). Most notably, I did not find *M. obligatum* nor *P. aenigmaticus* in any of the seed cultures.

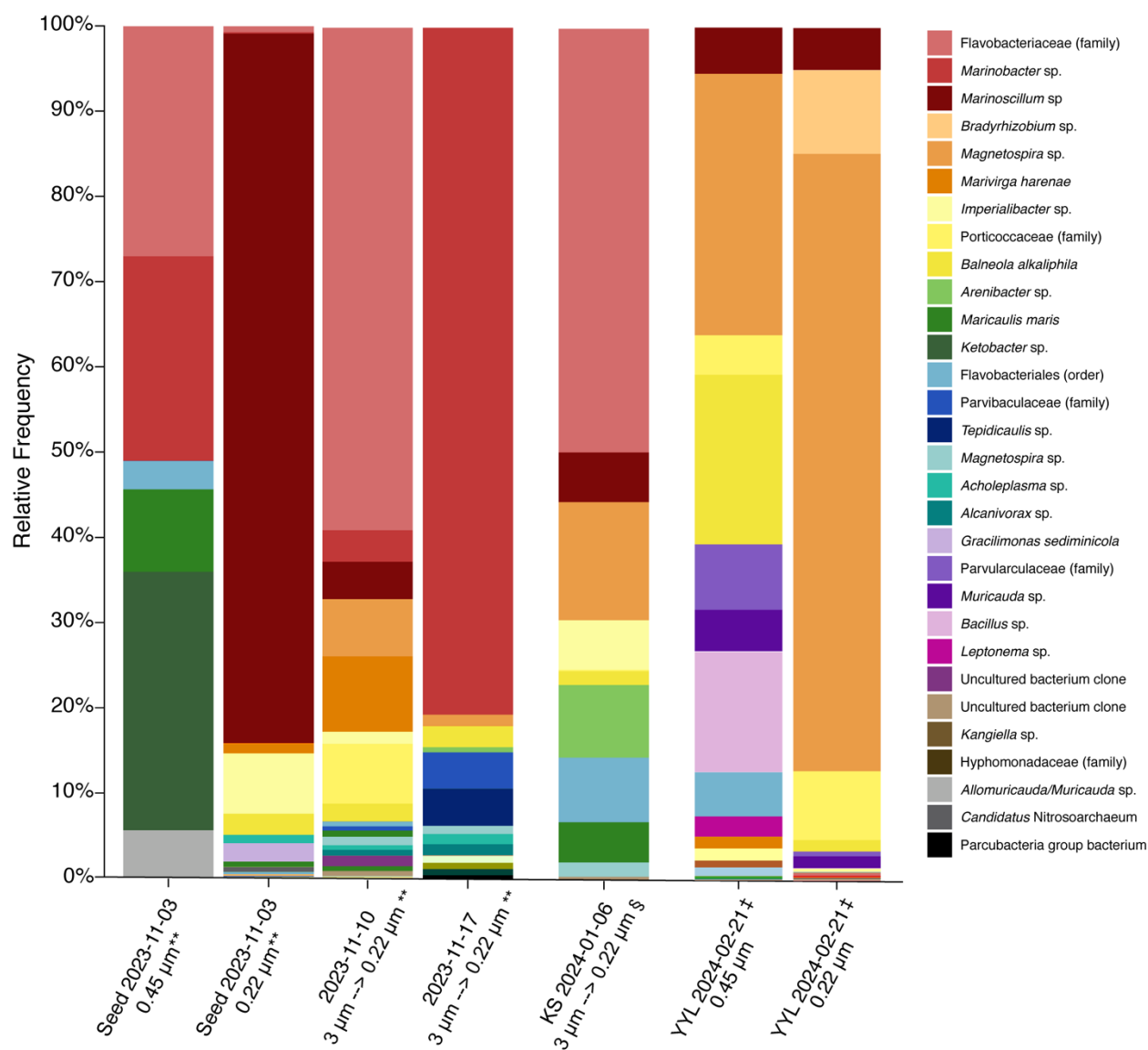


Figure 3.12 Relative abundance plots of bacterial communities associated with various seed cultures of *N. oceanica* IMET1. Samples from the same seed culture are clustered together and are marked with the same subscripted symbol. Details on experimental conditions are listed in Table 3.3. KS= seed culture supplied by Kieran Szelong of the Hill laboratory. YYL= seed culture supplied by Dr. Yi-Ying Lee of the Li laboratory.

Table 3.3 Sample details for Figure 3.12. All samples were *N. oceanica* IMET1 and samples with the same superscripted symbol are from the same culture, albeit different experimental conditions. LJ= Lauren Jonas, KS= Kieran Szelong, YYL= Dr. Yi-Ying Lee.

Sample name	Culture conditions	Origin of culture
Seed 2023-11-03 0.45 μm^{**}	Seed culture	Hill Lab – LJ
Seed 2023-11-03 0.22 μm^{**}	Seed culture	Hill Lab – LJ
2023-11-10 3 $\mu\text{m} \rightarrow 0.22 \mu\text{m}^{**}$	Bubbled with 10% CO ₂ for 2 weeks	Hill Lab – LJ
2023-11-17 3 $\mu\text{m} \rightarrow 0.22 \mu\text{m}^{**}$	Bubbled with 10% CO ₂ for 2 weeks	Hill Lab – LJ
KS 2024-01-06 3 $\mu\text{m} \rightarrow 0.22 \mu\text{m}^{\S}$	Bubbled with 10% CO ₂ for 3 weeks	Hill Lab – KS
YYL 2023-02-21 0.45 $\mu\text{m}^{\ddagger}$	Bubbled with 10% CO ₂ for 18 days	Li Lab – YYL
YYL 2023-02-21 0.22 $\mu\text{m}^{\ddagger}$	Bubbled with 10% CO ₂ for 18 days	Li Lab – YYL

3.5 Discussion

3.5.1 Bacterial communities associated with *T. obliquus* at 1 L scale

The three most abundant bacterial taxa associated with *T. obliquus* cultures were *Algoriphagus aquaeductus*, *Erythrobacter* sp., and *Allorhizobium* sp., all of which have been previously reported as bacterial symbionts of microalgae. *Algoriphagus* and *Porphyrobacter*³ were dominant genera within the microbiome of the microalgae *Ochromonas* sp. and *Chlorella* sp. along with the genus *Rhizobium* (Zhang *et al.*, 2021). The *Algoriphagus* genus has also been found to be associated with *N. oceanica* (Ling *et al.*, 2020). Interestingly, *Porphyrobacter* spp. were found to be the most dominant organisms associated with both *T. obliquus* HTB1 cultures bubbled with flue gas (10-12% CO₂) and bubbled with air a decade ago by Dr. Ryan Powell, a former PhD student of the Hill Laboratory (Powell, 2014). *Porphyrobacter* spp. comprised 56% of the bacterial sequences found in *T. obliquus* cultures grown with flue gas and 85% of the sequences of those grown with air (Powell, 2014). The *Porphyrobacter* genera has also been isolated from a cyanobacterial-microbial consortium (isolate HT-58-2) (Hughes *et al.*, 2018). A fifth bacterial species of significance is *Lacibacter* sp. (*cauensis*), which only appears in the bacterial communities closely associated with *T. obliquus* grown bubbled with 10% CO₂ (Figure 3.4). This taxon could be the contributing species that causes the significant difference in beta diversity found between the CO₂ and Air treatments in the 0.45 µm fraction (p-value = 0.002, Table 3.2). As *T. obliquus* HTB1 grew better (as shown by OD, cell count and dry weight measurements) when bubbled with 10% CO₂ compared with air only (Figure 3.3), *Lacibacter cauensis* could be contributing positively to microalgal growth. However, no growth promotion

³ The genera *Erythrobacter*, *Porphyrobacter* and *Erythromicrobium* all belong to the family Erythrobacteraceae and are often misclassified as the same genus (Lee *et al.*, 2005).

effects have previously been reported for *Lacibacter* sp. and microalgae; this possibility warrants further investigation. One study reported that *Lacibacter* sp. increased in relative abundance in both free-living and particle-attached communities after a microalgal consortia was treated with a cyanobactericidal compound, paucibactin A (Le *et al.*, 2024). Regardless of treatment with air or 10% CO₂, closely associated bacterial communities (0.45 µm fraction) were distinct from free-living bacterial communities (0.22 µm fraction) (Table 3.2), suggesting that communities form distinct spatial relationships within the microalgal phycosphere while remaining relatively stable under different experimental treatments.

3.5.2 Bacterial communities of *N. oceanica* at 1 L scale

A previous study pursuing wastewater treatment using microalgal-bacterial granular sludge, found that the wastewater treatment was enhanced with addition of NaHCO₃ that created a more favorable environment for Proteobacteria, Chloroflexi, and Cyanobacteria (Shi *et al.*, 2024). *N. oceanica* grew better as shown by OD, cell count and dry weight measurements with addition of 0.02 M NaHCO₃ regardless of whether the cultures were bubbled with air (Figure 3.5 A-D) or CO₂ (Figure 3.5 E-H). However, neither Chloroflexi nor Cyanobacteria were found in our system. I was unable to conduct a statistical analysis comparing beta diversity of bacterial communities of *N. oceanica* bubbled with air vs. bacterial communities bubbled with CO₂ because they were grown in two separate experiments. However, all five of the dominant species found within the communities bubbled with air—*P. aenigmaticus*, *M. obligatum*, *M. maris*, *Gracilimonas sediminicola*, and *M. pacifica* (Figure 3.8)—were found months later during the experiment using 10% CO₂ (Figure 3.9). The bacterial communities of the microalgae bubbled with 10% CO₂ did have more species (a higher alpha diversity) than those with air. Among the

bacterial groups found to be associated with *N. oceanica* IMET1, *M. maris* (previously described in the *Caulobacter* genera) is a common aquatic and halophilic species that has been described as scavenger of carbon in oligotrophic habitats (JGI Genome Portal; Abraham *et al.*, 1999). *B. alkaliphila*, *M. pacifica*, and *G. sediminicola* all belong to genera that have been previously found within a consortium of microorganisms capable of precipitating CaCO₃ (Skorupa *et al.*, 2019). *Gracilimonas* spp. (of the order Balneolales) have been recovered from various hypersaline soda lakes and CaCO₃ formations (Vavourakis *et al.*, 2016; Sumrall *et al.*, 2017; Zhilina *et al.*, 2023). This finding is relevant as CaCO₃ precipitation has been a goal of our laboratory (Jonas *et al.*, 2025a). Other notable symbionts of IMET1 were *R. spongicola*, *Marivirgia* sp., and an uncultured Gammaproteobacterium. The bacterial community associated with *N. oceanica* IMET1 has been analyzed once previously with Alphaproteobacteria (48%) and Bacteroidetes (28%) dominating the culturable bacterial community (Wang *et al.*, 2012).

3.5.3 Community composition changes within seed cultures, filtration methods, and scale up

When scaling up the system to 500 L and 6,800 L, the cultures were grown at Hy-Tek Bio's site at the Back River Waste Water Treatment of Baltimore. The seed cultures were provided by the Li and Chen laboratories at IMET, and algal growth media was prepared on site and not autoclaved. This could explain why new taxa appeared at the larger scales with some bacteria persisting within the community (Figures 3.10 and 3.11). At the 6,800 L scale, *T. obliquus* was grown for a few weeks before the bioreactor failed (Appendix, Figure A3.8). A growth experiment was planned for this bioreactor where samples for microbiome analysis

would be taken regularly. Because of the bioreactor crash, this experiment did not take place, however one sample was taken before the incident (Figure 3.11). There were no common bacteria shared between *T. obliquus* grown at the 6,800 scale with that of the cultures grown at the 1 L and 500 L scales. This could be due to the fact that tap water was used to make the microalgal growth medium in the large-scale experiment which may have allowed for new bacteria to overtake the communities present in the *T. obliquus* seed culture.

For *N. oceanica*, only two bacteria were found at both the 1 L and 500 L scale, *P. aenigmaticus* and *M. obligatum*. At the time of discovery, these bacteria were unclassified and were assigned the name ‘uncultured bacterium clone’. Further analysis revealed them to be members of the phylum Patescibacteria. The 16S rRNA gene sequences from the 500 L dataset were identical to those from the 1 L dataset. This finding prompted our interest in these bacteria and was the impetus for metagenomic sequencing discussed in Chapter 4. Intriguingly, *P. aenigmaticus* and *M. obligatum* were also found in the *T. obliquus* cultures in the 500 L photobioreactor at Hytek Bio (Figure 3.10) but were not found in *T. obliquus* cultures when grown at the 1 L scale at IMET (Figure 3.4). *Allorhizobium* sp. was the third bacterium found at both 1 L and 500 L scales in *T. obliquus* cultures. This bacterium belongs to the Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium clade, a well-studied group of organisms that are symbionts of legumes in their root nodules (De Lajudie *et al.*, 1998). This clade has been found to be associated with microalgae such as *Chlorella sorokiniana* (Steichen *et al.*, 2020) and *Auxenochlorella pyrenoidosa* (Li *et al.*, 2025). Genes encoding IAA synthesis have been found in the genome of *Rhizobium* sp. associated with the microalga, *Chlorococcum robustum*, which may indicate potential microalgal growth promotion (Geng *et al.*, 2024). The growth promoting

effects of *Allorhizobium/Rhizobium* sp. mediated by IAA and vitamin B12 have been reported previously in microalgae (Do Nascimento *et al.*, 2013) and plants (Bhattacharyya and Jha, 2012).

There are several biases that are important to note when interpreting microbiome data, for example, abundances are relative (not absolute), certain taxa may have primer and amplification biases, and that the observed communities are a snapshot of the true underlying abundances throughout the experiment. All studies that discriminate closely associated and free-living bacteria rely on size fractionation by filtration. This can lead to an overestimation of attached bacteria due to trapping of some free-living bacteria on the 0.45 μm filters (Riemann *et al.*, 2000). These biases could contribute to the differences I see between various seed cultures of *N. oceanica* (Figure 3.12). The seed cultures were continuously analyzed in an attempt to find *M. obligatum* and *P. aenigmaticus*. DNA yields were often low in 0.22 μm filter fraction and ethanol precipitations were needed to recover amplifiable concentrations. To help increase DNA yields, I first filtered cultures through a 3 μm filter with the intention of having more bacteria pass through than if I were to use a 0.45 μm filter. I did this multiple times and pooled the filtrate to then capture bacterial cells on a 0.22 μm filter. The first four bars in Figure 3.12 are all the same seed culture filtered in different ways. Although there are some consistent taxa throughout, namely *Marinobacter* sp. and *Marinoscillum* sp. and a member of the Flavobacteriaceae family, the latter two samples have more bacteria at low relative abundance. The seed cultures from Kieran Szelong and Yi-Ying Lee also come with distinct bacterial taxa that are not found in the seed culture grown by me, *i.e.* *Arenibacter* sp. and *Muricauda* sp. (Figure 3.12).

3.5.4 Consistencies and dominance of two Patescibacteria

Within the *N. oceanica* seed culture dataset (Figure 3.12), there are some taxa present that were also found within the *N. oceanica* community grown with air and CO₂ (Figures 3.8 and 3.9): *B. alkiliphila*, *M. maris*, *G. sediminicola*, *Marinoscillum* sp., *Mycobacterium* sp., *Leptonema* sp., and *Marivirgia* sp. These bacteria are consistently present in *N. oceanica* cultures with the exception of the one sample taken from the 500 L bioreactors. Another consistency throughout the microbiome analysis of both *T. obliquus* and *N. oceanica* was the dominance of the two aforementioned Patescibacteria, *P. aenigmaticus* and *M. obligatum*. These two Patescibacteria were found to be associated with two microalgae at two salinities—the freshwater strain, *T. obliquus* HTB1 and the marine strain, *N. oceanica* IMET1—at two different scales (1 liter and 500 liters), within two different filter size fractions (0.45 µm and 0.22 µm), and regardless of whether cultures were bubbled with ambient air, 5% CO₂, or 10% CO₂. Metagenomic sequencing was performed after these findings with the aim of recovering genomes for the two Patescibacteria of interest (Chapter 4) and then detailed annotation was performed to elucidate their potential function (Chapter 5). There was a ‘Parcubacterium group bacterium’ recovered in the dataset shown in Figure 3.12; however, this bacterium was not *P. aenigmaticus* or *M. obligatum*. This could potentially indicate there are more novel Patescibacteria that could be recovered from our microalgal cultures.

Chapter 4. Construction and analysis of metagenomic assembled genomes (MAGs) and nomenclature of two novel species, two novel genera, one novel family, and one novel order

4.1 Abstract

In the previous chapter, I describe the discovery of two bacteria with no classification beyond the phylum level (Patescibacteria) that were abundant symbionts of both *Tetradasmus obliquus* HTB1 and *Nannochloropsis oceanica* IMET1 across various experimental conditions. Because of this striking dominance, I used metagenomic sequencing and recovered seven high quality metagenomic assembled genomes (MAGs), two of which belonged to the Patescibacteria of interest. I then used various metrics suggested by experts in the field and worked closely with the SeqCode to establish taxonomic novelty and assign names for two novel species belonging to two novel genera, one novel family, and one novel order. In this chapter, I present two novel species under the Seqcode (seqco.de/r:ywe1blo2), *Phycocordibacter aenigmaticus* and *Minusculum obligatum* gen. nov. sp. nov. I subsequently present the genus *Phycocordibacter* that serves as the nomenclatural type of the family Phycocordibacteraceae and the order Phycocordibacterales. Both *M. obligatum* and *P. aenigmaticus* were recovered from cultures of both microalgae and possess features typical of Patescibacteria, such as reduced genomes (<800k bp). Five other high-quality MAGs were recovered: two from *N. oceanica* IMET1 cultures (*Gracilimonas sediminicola* & *Microcella pacifica*) and three from *T. obliquus* HTB1 cultures (*Algoriphagus aquaeductus*, *Erythrobacter* sp., and *Allorhizobium* sp.). All MAGs underwent phylogenetic analysis with the alignment of the 16S rRNA gene and genomes were submitted to the National Center for Biotechnology Information (NCBI). Genome descriptions as well as the taxonomic novelty of *M. obligatum* and *P. aenigmaticus* have been published (Jonas *et al.*, 2025b [in review]).

4.2 Introduction

4.2.1 Assembly of metagenome-assembled genomes (MAGs) from long-read sequencing data

While analysis of the 16S rRNA gene provides useful information into relative abundances and diversity of bacterial communities, metagenomic sequencing is a superior strategy that provides information from all DNA within a sample. Barcoding analysis relies on PCR to amplify specific genetic regions (e.g., 16S rRNA for bacteria), which limits the length of reads and often can result in sequencing chimeras (Y. X. Liu *et al.*, 2021). Metagenomic sequencing, on the other hand, involves direct sequencing of all DNA in a sample, albeit at a higher cost and computational demand. Genome-centric metagenomics results is a subset within bioinformatic analysis in which metagenomic sequencing reads are assembled into contigs and binned into groups to form metagenomic-assembled genomes (MAGs). This dissertation used long read sequencing technology, namely the MinION and GridION sequencers (Oxford Nanopore) which can generate standard reads of 1–60 kb (kb = thousand bp) and ultra-long reads of >100 kb – >1Mb (Mb = million bp) (Damaraju *et al.*, 2024). However, sequencing ultra-long reads is limited by the quality and fragmentation of the original DNA extract. Recent efforts in long read metagenomics sequencing have allowed for the reconstruction of prokaryotic genomes, many of which make up what is known as “microbial dark matter”. It has been estimated that >99% of microbes have yet to be formally named, leading to vague and clunky alphanumeric labels dispersed across the prokaryotic tree of life (Sutcliffe *et al.*, 2021). The description of novel taxonomic groups is significant not only to fill in these gaps but also can unveil unusual biological traits. One example is the hyper-reduced genome size and metabolic capacity of many members of the vastly uncharacterized phylum, Patescibacteria.

4.2.2 Microbial dark matter and the nomenclature of uncultured prokaryotes

Microbial dark matter refers to various candidate phyla that make up an assemblage of bacteria that are unable to be axenically cultured. The Great Plate Count Anomaly is a long-standing estimation that <1% of bacterial species have been cultivated in the laboratory (Staley and Konopka, 1985) and it has been estimated that less than half of currently recognized bacterial phyla include cultivated representatives (Rappé and Giovannoni, 2003). These findings have been compounded by culture-independent sequencing and the rapidly growing collection of environmental MAGs that would otherwise be undetectable with traditional laboratory techniques. In response to the increasing number of MAGs that have no taxonomic classification, there have been recent calls to implement new nomenclature standards, particularly the designation of type strains. Type strains traditionally do not officially extend to uncultured microorganisms as governed by the International Code of Nomenclature of Prokaryotes (ICNP)⁴ (Oren *et al.*, 2023). In 2020, a proposal to allow the use of gene sequences instead of cultivated organisms as type material for the naming of novel prokaryotes was rejected by the ICNP (Sutcliffe *et al.*, 2020). When naming uncultivated prokaryotes, the provisional status ‘*Candidatus*’ can be used, although the nomenclature of *Candidatus* taxa is not formally recognized by the ICNP as of yet. There are several issues commonly associated with nomenclature of uncultured prokaryotes, including linguistic errors, absence of an etymology for the name, the failure to designate the nomenclatural type, and naming of higher taxa without naming the type genus and species (Oren, 2017). Recently, Chuvochina and colleagues (2019)

⁴ Formerly the *International Code of Nomenclature of Bacteria* (1966), and, earlier, the *International Code of Nomenclature of Bacteria and Viruses* (1958) and the *International Bacteriological Code of Nomenclature* (1948). Also known as the *Bacteriological Code*, and, since 2008, as the *Prokaryotic Code*.

provided case studies for designating metagenomes as type material for the naming of higher taxonomic ranks. The INCP Rules 8 and 9 state that the names of the highest categories up to the rank of class are based upon the designation of type genus, and derived from the stem of its name with the addition of the certain rank suffixes, for example ‘-aceae’ for the rank of family and ‘-ales’ for the rank of order (Parker *et al.*, 2019). Recently, the nomenclature of the phylum rank was changed in that the suffix ‘-ota’ will be added to the name of one of the subordinate classes (Oren *et al.*, 2015; Whitman *et al.*, 2018). The name of the class is based on a specific type genus, *i.e.* the phylum Acidobacteriota and class Acidobacteria is named from the type genus *Acidobacterium* (Kishimoto *et al.*, 1991). Consequently, when designing novel nomenclature, one only needs to name a genus to provide the basis for all higher taxonomic ranks. Therefore, researchers have been strongly encouraged to name at least one species and genus within any newly delineated uncultured lineage based on high quality MAGs recovery (Chuvochina *et al.*, 2019). Designating this type material ensures nomenclature stability even if the phylogenetic position of newly discovered lineages may change (Parks *et al.*, 2018a).

4.2.3 Validating taxonomic novelty and establishing nomenclature from sequence data

Traditionally, microbial taxonomy has relied on descriptions of morphology and metabolic profiles to differentiate and classify novel microorganisms. However, uncultivated organisms are often only analyzed *in silico* without the ability for the organisms to be visualized or enriched. Taxonomic novelty must instead be established through various bioinformatic analyses such as overall genomic relatedness indices (OGRIs) (Chun and Rainey, 2014). Two common OGRIs are average nucleotide identity (ANI) and digital DNA–DNA hybridization

(dDDH). Species are often defined as having an ANI of 95-96% and a dDDH of 70% (Richter and Rosselló-Móra, 2009; Meier-Kolthoff *et al.*, 2013). Alternatives and supplementations to traditional species delineation metrics like dDDH include average amino acid identity (AAI) and multigene-based phylogenies (MBPs). MBPs rely on an alignment of a large number (often 120) concatenated universal bacterial genes (Glaeser and Kämpfer, 2015). Regardless of the chosen metrics, researchers are urged to combine various methods and only work with MAGs that have appropriate sequencing depth (also known as coverage) (Riesco and Trujillo, 2024).

When coming across a potential new species in sequence data, one must first validate its taxonomic novelty using the metrics described above. To then designate the species as a type strain, the genome sequence (along with novelty validation) must be submitted to the Code of Nomenclature of Prokaryotes Described from Sequence Data (SeqCode). The Seqcode was formed in response to the 2020 rejection of the proposal that DNA sequences could serve as nomenclatural types and be incorporated into the existing ICNP rules (Konstantinidis *et al.*, 2017; Murray *et al.*, 2020). The Seqcode enables valid publication of names of prokaryotes using DNA sequences from isolate genomes, MAGs or single-amplified genome sequences (SAGs) as the nomenclatural types (Hedlund *et al.*, 2022; Whitman *et al.*, 2022). All data is publicly available within the SeqCode Registry, a website in which names and nomenclatural types are registered, validated by SeqCode staff and linked to metadata (Whitman *et al.*, 2022). While names can be published and then registered with the SeqCode, it is recommended to validate names before publication. A nomenclatural curation team confirms taxonomic novelty and reviews the formation of Latin names before the submission of a manuscript, a process fairly like manual nomenclatural checks during peer review at the International Journal of Systematic and Evolutionary Microbiology (IJSEM) (Whitman *et al.*, 2022).

4.2.4 Overview of this chapter

In this chapter, I describe the details of five rounds of metagenomic sequencing conducted by me using the MinION and GridION platforms available in the BioAnalytical Services Laboratory (BASLab) at IMET. I describe the genome characteristics and phylogenies of seven MAGs that were recovered from these sequencing runs, paying particular attention to two MAGs that had taxonomic classification only to the class rank. Using the SeqCode, I have formally named these bacteria. Here I present *Phycocordibacter aenigmaticus*, a novel species within the genus *Phycocordibacter* gen. nov., which serves as the nomenclature type for the family Phycocordibacteraceae fam. nov. and the order Phycocordibacterales ord. nov. The second bacterium, *Minusculum obligatum*, is within the genus *Minusculum* gen. nov. and falls within the same family and order. Novelty was determined through ANI, AAI, dDDH, a MBP tree, and protologued (Chuvochina *et al.*, 2019; Whitman *et al.*, 2022). The same seven MAGs described here will be further analyzed in Chapter 5 when discussing functional profiling and metabolic reconstruction.

4.3 Methods

4.3.1 Metagenomics library preparation and sequencing

DNA concentrations were checked by absorbance at 260 nm using a UV–visible spectrophotometer (Nanodrop) and fluorometry (Qubit 1X dsDNA Assay Kit + Qubit fluorometer) and adjusted to the ideal starting quantity of 100-200 fmol (~1000 ng) in 47 µl nuclease-free water. DNA was prepared into sequencing libraries using the Ligation Sequencing Kit V14 (Oxford Nanopore) following the manufacturer’s protocol with the following adjustments: during the last step of both the End-Prep and Adapter Ligation and Clean-up protocols, tubes containing AMPure XP beads and the end-prep reaction were incubated at 37°C for 2 minutes before eluate was removed. Libraries were made up to 12 µl at 10-20 fmol and loaded onto the GridION sequencing platform (Oxford Nanopore) strictly following the manufacturer’s protocol. For the second round of sequencing, the sample was put through the Short Read Eliminator (SRE) kit (PacBio). This protocol is designed to significantly enhance mean read length by depleting short DNA fragments and DNA is purified through standard ethanol precipitation techniques to remove contaminants for difficult samples. The number of reads obtained was 71,791 and 232,714,605 total bases were sequenced (Table 4.1- ‘MAG2’). Compared to MAG1 Round1 with 434,869 reads and 1.43 billion base-called base pairs (Table 4.1), this run was of subpar output and was not selected for further analysis. The Short Read Eliminator was not used for subsequent samples.

4.3.2 Assembly of Metagenomic Assembled Genomes (MAGs)

Reads recovered were visualized for length and quality using Nanoplot and filtered using Nanofilt (Figure 4.1) (De Coster and Rademakers, 2023). Adapter sequences were removed with

Porechop and sequences were assembled using Flye (v2.9.3) twice: once using the metagenomics parameter (--meta) and once without (Kolmogorov *et al.*, 2019). The best assemblies were considered those with the lowest number of contigs and the highest N50 value. The N50 is the length of the median contig, meaning half of the contigs in the full assembly are that length in base pairs or longer. Coverage was visualized with the Integrative Genome Viewer (IGV) by overlaying the sorted.bam file on top of the assembly.fasta output (Thorvaldsdóttir *et al.*, 2013; Robinson *et al.*, 2023). Circular contigs with high coverage were extracted for annotation and checked for completeness using CheckM2 (Chklovski *et al.*, 2023). Adapting an existing KBase narrative (Chivian *et al.*, 2023), MaxBin2 (v2.2.4) and CONCOCT (v1.1) were used for binning (Alneberg *et al.*, 2014; Wu *et al.*, 2016), RASTtk (v1.073) for annotating multiple microbial assemblies (Brettin *et al.*, 2015), and GTDB-Tk (v2.3.2) for taxonomic classification (Chaumeil *et al.*, 2020). Bins that were >80% complete with <10% contamination were selected for further analysis. Assemblies were extracted from binned contigs within KBase and were uploaded to the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) (Olson *et al.*, 2023). Fasta files of circular contigs from the original Flye assembly were also uploaded. The Genome Annotation Service uses the RASTtk tool kit to annotate bacterial genomes using the genome of the closest relative (Brettin *et al.*, 2015). MAGs were deposited in the NCBI database under the following accession numbers:

Phycocordibacter aenigmaticus: CP174359

Minusculum obligatum: CP174360

Gracilimonas sediminicola: CP175953

Microcella pacifica: JBJNZ000000000

Algoriphagus aquaeductus: JBJINY000000000

Allorhizobium sp.: JBJIOA000000000

Erythrobacter sp.: JBJIOB000000000.

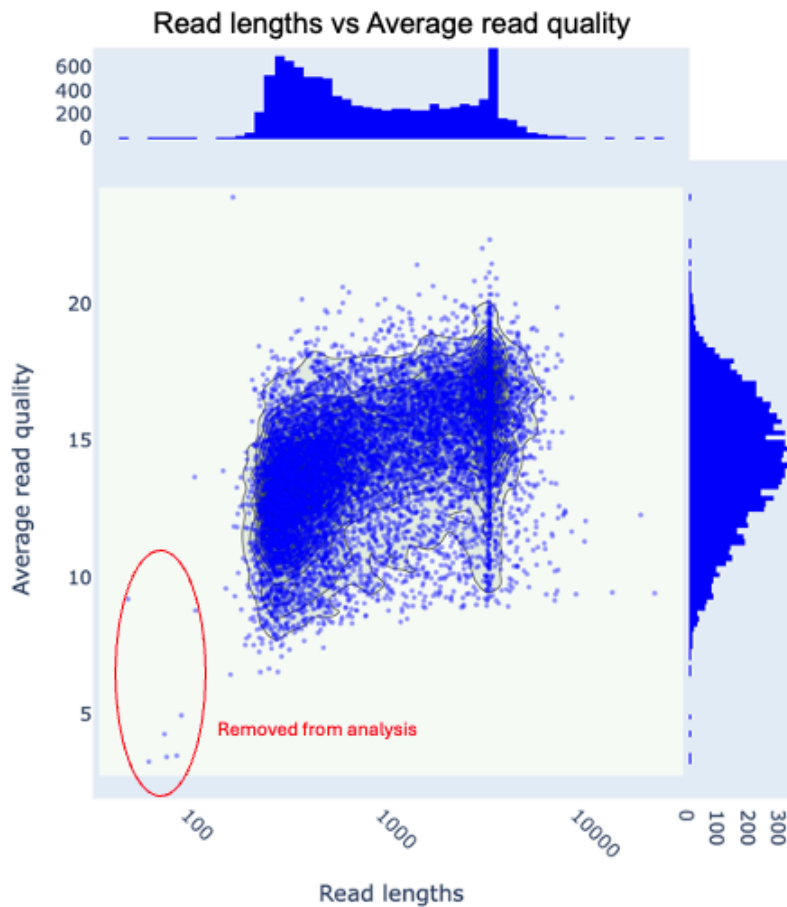


Figure 4.1. Example output of the bioinformatic program, Nanoplot, from sequencing round, ‘MAG Round 3’. Average read quality (Phred quality score) vs. Read lengths (in base pairs, bp) of raw sequences. Sequences in red circle were removed from the analysis using NanoFilt due to both low quality and length (<Phred 10, <100 bp).

4.3.3 Phylogenetic analysis

Phylogenetic trees were constructed from partial sequence (~1,147 bp) of the 16S rRNA gene. The full 16S rRNA gene sequence from each MAG was aligned with that of nearest relatives using NCBI's BLASTn program. Nearest relatives were aligned with MUSCLE (5.1) (30) and manually trimmed to equal length to ensure accurate phylogenetic analysis. Inconsistent lengths can lead to misalignment and consequently miscalculated tree construction. BLAST nearest-relative alignment, MUSCLE alignment, and trimming described above were all done using the Geneious Prime software (2024.0.5) (31). The alignments were then uploaded into the Méthodes et algorithmes pour la bio informatique (MAB) in NGphylogeny.fr (32, 33). This program verifies the original alignment, again using MUSCLE. The alignments moved forward with a curation by Gblocks which eliminates poorly aligned positions and divergent regions (34), PhyML for phylogeny (35–37), and TreeDyn for tree rendering (38). For confidence analysis, data was resampled using 1,000 bootstrap replicates. Branches with < 50% bootstrap values were collapsed within the tree. FigTree (v.1.4.4) (39) and Adobe Illustrator were used for visualization. 16S rRNA gene phylogenetic analysis based on sequence extracted from genomes was also carried out with the Type Strain Genome Server (TYGS) and programs therein (40). Multigene-based phylogenetic trees were inferred using 120 single-copy concatenated protein markers using the GTDB-Tk Classify workflow, the Interactive Tree of Life (iTOL) online tool, and Adobe Illustrator (41).

4.3.4 Novelty determination and nomenclature

MAGs were established as high-quality by following the Minimum Information about a Metagenome-Assembled Genome (MIMAG) criteria and standards set forth by experts in the

field (Chuvochina *et al.*, 2019; Riesco and Trujillo, 2024). Size, L50 values, N50 values, coverage, and GC ratio was determined by Flye assembly. The Microbial Genomes Atlas (MiGA v1.2.60) was used to determine genome distance metrics based on amino acid identity (AAI) and average nucleotide identity (ANI) against the TypeMat database (Rodriguez-R *et al.*, 2018). TYGS was used to calculate digital DNA hybridization (dDDH) values. These metrics, along with relative evolutionary divergence (RED) scored provided by GTDB-Tk, uploaded to the SeqCode in Validation Path 1 (seqco.de/r:ywe1blo2.) (Hedlund *et al.*, 2022; Whitman *et al.*, 2022). Nomenclature was proposed based on MAG features and relation to microalgae.

4.4 Results

Table 4.1 Organizational map of tables and figures of this chapter. Clickable links direct reader to data of interest.

Organism	Raw sequencing statistics	Post filtering statistics	Genome statistics and quality	Circular genome visualization	Phylogenetic trees
<i>Minusculum obligatum</i>	Table 4.2	Table 4.3	Table 4.4	Figure 4.4	Figure 4.5 , Figure 4.6 , Figure 4.17
<i>Phycocordibacter aenigmaticus</i>	Table 4.2	Table 4.3	Table 4.4	Figure 4.2	Figure 4.3 , Figure 4.6 , Figure 4.17
<i>Microcella pacifica</i>	Table 4.2	Table 4.3	Table 4.4	Figure 4.9	Figure 4.10
<i>Gracilimonas sediminicola</i>	Table 4.2	Table 4.3	Table 4.4	Figure 4.7	Figure 4.8
<i>Algoriphagus aquaeductus</i>	Table 4.2	Table 4.3	Table 4.4	Figure 4.11	Figure 4.12
<i>Erythrobacter</i> sp.	Table 4.2	Table 4.3	Table 4.4	Figure 4.13	Figure 4.14
<i>Allorhizobium</i> sp.	Table 4.2	Table 4.3	Table 4.4	Figure 4.15	Figure 4.16

4.4.1 Details and statistics of sequencing rounds using the Oxford Nanopore

MinION and GridION sequencer

In total, six rounds of long read metagenomic sequencing were performed. Four rounds recovered MAGs while two were deemed unsuccessful and not pursued for further analysis. The first two sequencing rounds, ‘MAG1_Round1’ and ‘MAG1_Round2’ (DNA from IMET1 cultures) were performed on the Oxford Nanopore MinION platform and the remaining four runs, ‘MAG2’ (IMET1), ‘MAG3’ (IMET1), ‘MAG4’ (HTB1), and ‘MAG5’ (HTB1) were sequenced on the GridION platform (Table 4.2). The different sequencing platforms contain the same core components and were not expected to alter the quality of results. However, the sequencing output did improve significantly over time due to refining the techniques needed for

DNA library preparation and loading. For example, ‘MAG3’ and ‘MAG5’ recovered approx. 5 - 5.4 billion base pairs whereas ‘MAG1 Rounds 1 & 2’ recovered approx. 1 billion base pairs (Table 4.2).

Nanoplot is a tool designed to visualize long read sequencing data and is useful in deciding filtering metrics, such as length minimums and quality cutoffs (De Coster and Rademakers, 2023). Table 4.3 shows the improved quality of reads when filtered with NanoFilt (De Coster and Rademakers, 2023) and when Nanopore-specific adapters were removed with Porechop (Wick and Volkening, 2018). Figure 4.1 shows an example of a Nanoplot that was used to determine which sequences were kept for further analysis. Raw reads from MAG Rounds 1 and 2 were combined into one analysis. These sequences and those from MAG3 and MAG5 were selected for further analysis based on the total bases sequenced and number of reads recovered. When short reads were filtered and adapters were removed, quality scores improved for both rounds of sequencing (Table 4.3). Seven metagenomic assembled genomes (MAGs) were extracted from these analyses after assembly with Flye.

Round 4 (Table 3.1- ‘MAG4’) was not selected for further analysis as it did not yield any metagenomes and had a poor output (195 Mb total base pairs and 29,201 reads) (Table 4.2). This was because the sequencer did not have a sufficient number of pores available. After a few hours, >50% of its available pores became unavailable (Appendix, Figure A4.1). Therefore, genomes of bacteria associated with HTB1 were recovered from a subsequent sequencing run, MAG5.

Table 4.2 Sequencing statistics of six rounds of Nanopore sequencing. Blue font indicates that MAGs were recovered from the dataset, and black font indicates both insufficient sequence quantity and quality for MAG recovery. Mb= million base pairs, Gb= billion base pairs. IMET1 = *Nannochloropsis oceanica* strain IMET1. HTB1 = *Tetrademus obliquus* strain HTB1.

Name	MAG1-Round1	MAG1-Round2	MAG2	MAG3	MAG4	MAG5
Sequencing platform	MinION	MinION	GridION	GridION	GridION	GridION
Total bases	1.43 <i>Gb</i> 1,427,925,984	918 Mb 918,015,821	23.3 Mb 232,714,605	5.45 <i>Gb</i> 5,446,122,139	195 Mb 195,271,607	5.14 <i>Gb</i> 5,139,911,566
Number of reads	434,869	269,260	71,791	3.87 Mb 3,855,281	29,201	2.46 Mb 2,461,737
Quality (mean)	10.7	10.9	14.4	12.9	14.5	12.8
Quality (median)	15.8	16.8	16.3	14.3	16.5	14.2
Length (mean, bp)	3,283.6	3,409.4	3,241.6	1,408.6	6,687.2	2,087.9
Length (median, bp)	2,979	3,586	3,583	710.0	5,740	1,481.0
N50 Read length (bp)	3,747	3,590	3,587	2,910	10,031	3,579
MAGs recovered	Yes	Yes	No	Yes	No	Yes
Microalgal culture	IMET1	IMET1	IMET1	IMET1	HTB1	HTB1

Table 4.3 Statistics of successful sequencing rounds before and after filtering for high quality, determining minimum length cut-offs, and removing Nanopore sequencing adapters. Mb= million base pairs, Gb= billion base pairs.

	MAG Rounds 1 and 2 combined		MAG Round 3		MAG Round5	
	Before processing (raw reads)	After removing seqs <100 bp & removing adapters	Before processing (raw reads)	After removing seqs <100 bp & removing adapters	Before processing (raw reads)	After removing adapters
Total bases	2.35 Gb 2,345,941,805	2.32 Gb 2,319,637,680	5.45 Gb 5,446,122,139	5.21 Gb 5,306,188,646	5.14 <u>Gb</u> 5,139,911,566.0	5.04 <u>Gb</u> 5,042,496,571.0
Number of reads	704,129	705,774	3.87 Mb 3,855,281	3.87 Mb 3,865,115	2.46 Mb 2,461,737.0	2.46 Mb 2,462,603.0
Quality (mean)	10.8	10.9	12.9	13.7	12.8	13.3
Quality (median)	16.2	16.6	14.3	15.3	14.2	15.1
Length (mean, bp)	3,331.7	3,286.7	1,408.6	1,372.8	2,087.9	2,047.6
Length (median, bp)	3,517	3,487	710	675	1,481.0	1,446
N50 Read length (bp)	3,594	3,542	2,910	2,922	3,579	3,529
Microalgal culture	IMET1		IMET1		HTB1	

4.4.2 Seven metagenome assembled genomes (MAGs)

In all, seven MAGs were assembled, two of the class Paceibacteria (Figures 4.2 and 4.4) as well as five others (Figures 4.7, 4.9, 4.11, 4.13, 4.15). Genome characteristics of all seven MAGs are shown in Table 4.4. All MAGs were characterized as high quality by the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) and Microbial Genomes Atlas (MiGA). According to the MIMAG standards (Bowers *et al.*, 2017)—Completion >90%; Contamination <5%; Presence of the 23S, 16S, and 5S rRNA genes and at least 18 tRNAs— five out of seven MAGs are considered high quality.

1. *Phycocordibacter aenigmaticus*

Phycocordibacter aenigmaticus gen. nov. sp. nov. consists of a single circular chromosome containing 721,362 bp and a GC content of 44.1% (Figure 4.2). Completeness and contamination were measured as 99.99% and 0.54% (Table 4.4). In total, 768 protein-coding sequences (CDS) were identified, with 528 (69%) of the predicted proteins having no detectable sequence similarity to other proteins in the SEED database used by RASTtk, and as such are annotated as “hypothetic proteins” (Overbeek *et al.*, 2014). The remaining 30% of sequences were given function assignments, with 120 proteins with Enzyme Commission (EC) numbers assignments, 94 proteins with Gene Ontology (GO) assignments, 80 proteins with Pathway assignments, 87 proteins with Subsystem assignments, 0 proteins with PATRIC genus-specific family (PLfam) assignments, 143 proteins with PATRIC cross-genus family (PGfam) assignments, and 0 proteins with Federally Integrated Genomics Families (FIGfam) assignments (Appendix, Figure A4.2).

The genome encodes for one 23S ribosomal RNA (rRNA), one 16S rRNA (1,465 bp bp), and one 5S rRNA (partial; 55% alignment). The genome also encodes 27 transfer RNAs (tRNAs), encoding for all amino acids except for asparagine. The 16S rRNA gene sequence has 100% percent identity with ‘uncultured bacterium clone 1’ recovered from the Illumina Miseq Platform when the 16S rRNA gene was amplified for prokaryotic barcoding analysis in Chapter 3 (Table 4.5, Figure 3.6 | Chapter 3).

Various phylogenetic trees were constructed to elucidate potential taxonomic placement. TYGS was used to create full length 16S rRNA gene trees only with neighbors that have been taxonomically classified (Figure 4.3). However, when working with highly unclassified lineages, it is more advantageous to construct trees with all available taxa, including formally named bacteria and those given alphanumeric codes together on one tree. A phylogenetic tree using a representative set of the bacterial sequences available in the NCBI database places both *Phycocordibacter aenigmaticus* and *Minuscolum obligatum* (discussed in next subsection) among numerous other uncultivated environmental bacteria (Figure 4.6). A second tree constructed with 120 concatenated single-copy universal bacterial genes (bac120) using the GTDBtk database provides contextual placement, showing that the two MAGs clearly fall within the Patescibacteria phylum (Figure 4.17A) and Paceibacteria class (Figure 4.17B). Accession number for *Phycocordibacter aenigmaticus* MAG: CP174359.

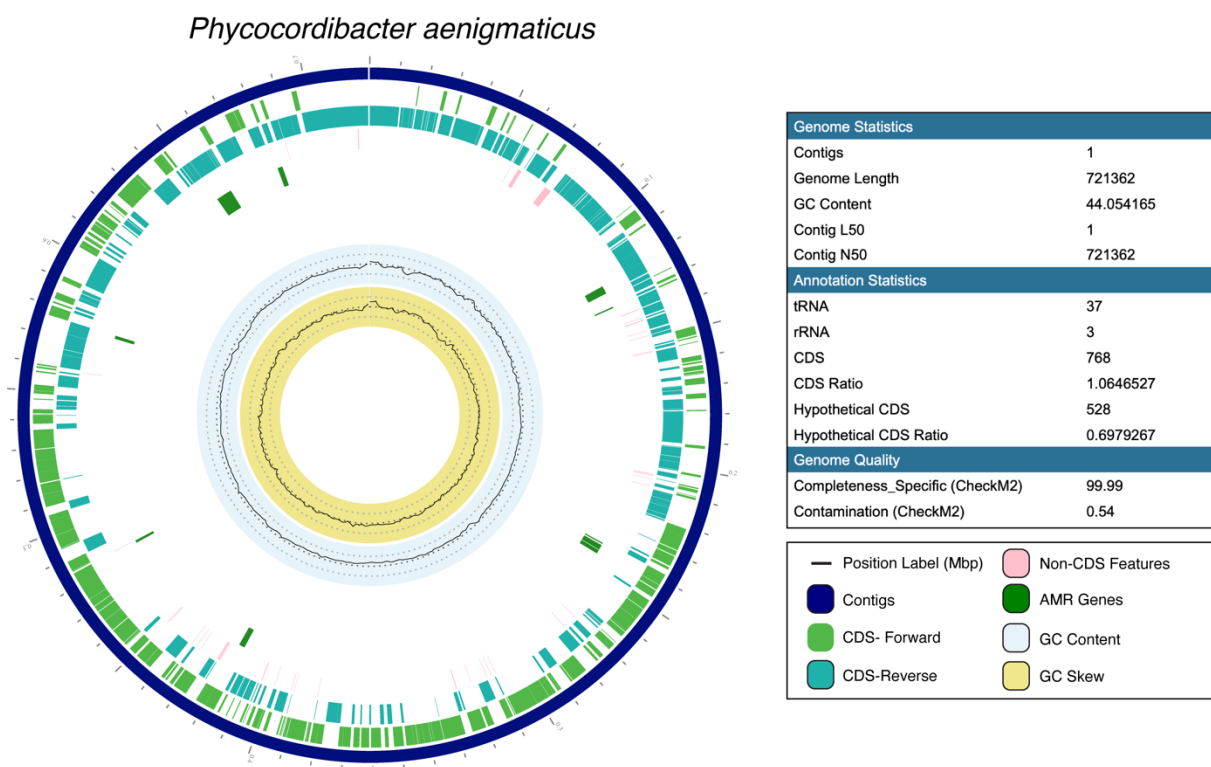


Figure 4.2 Circular genome view of *Phycocordibacter aenigmaticus*, a MAG recovered from cultures of *N. oceanica* IMET1 and *T. obliquus* HTB1. Genome visualization modified from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC). Genome statistics, annotation statistics, and genome completeness and quality are featured to the right. Figure displayed with permission from Jonas *et al.* (2025b).

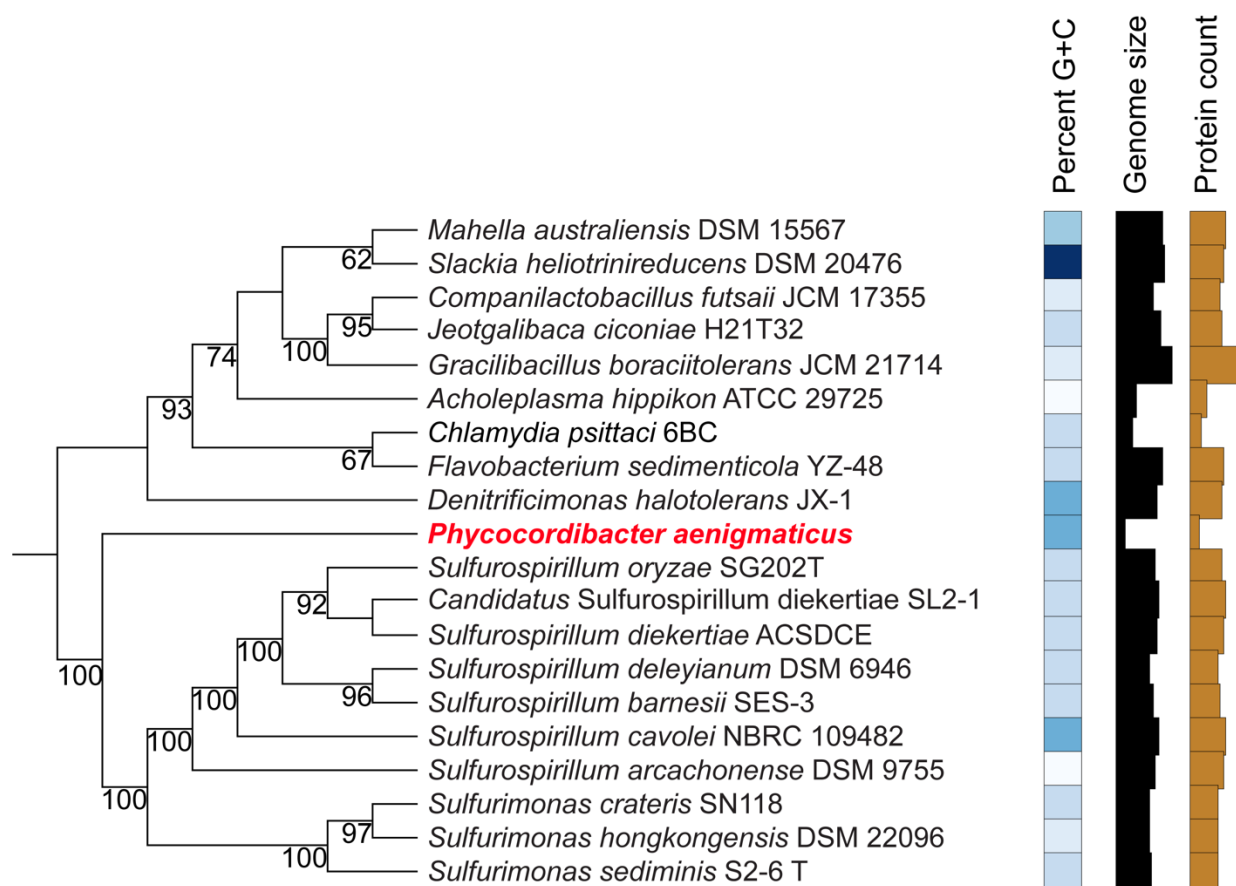


Figure 4.3 Phylogenetic inference of 16S rRNA genes taken from full genome sequences.

Phycocordibacter aenigmaticus was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm. An additional set of ten closely related type strains was determined via the 16S rDNA gene sequences. Distances were calculated using Genome BLAST Distance Phylogeny approach (GBDP).

2. *Minusculum obligatum*

Minusculum obligatum gen. nov. sp. nov. consists of a single circular chromosome containing 578,798 bp and a GC content of 43% (Figure 4.4). Completeness and contamination using CheckM2 were measured as 98.13% and 0.63% (Table 4.4). In total, 643 protein-coding sequences were identified, with 414 (64%) annotated as “hypothetic proteins” (Appendix, Figure

A4.3). (Overbeek *et al.*, 2014). The remaining 36% of sequences were given function assignments, with 115 proteins EC numbers assignments, 94 proteins with GO assignments, 76 proteins with Pathway assignments, 83 proteins with Subsystem assignments, 0 proteins with PLfam assignments, 128 proteins with PGfam assignments, and 0 proteins with FIGfam assignments (Appendix, Figure A4.3). The genome encodes for one 23S rRNA, one 16S rRNA (1,446 bp), and one 5S rRNA (partial; 77% alignment). The genome also encodes 35 tRNAs, encoding for all amino acids except for asparagine. The 16S rRNA gene sequence has 100% percent identity with ‘uncultured bacterium clone 2 recovered from the Illumina Miseq Platform when the 16S rRNA gene was amplified for prokaryotic barcoding analysis in Chapter 3 (Table 4.5, Figure 3.6 | Chapter 3).

TYGS was used to create full length 16S rRNA gene trees with neighbors that have been taxonomically classified (Figure 4.5). A phylogenetic tree using both *Phycocordibacter aenigmaticus* and *Minuscule obligatum* with a representative set of the bacterial sequences available in the NCBI database (Figure 4.6). A final tree (bac120) shows *Minuscule obligatum* within the Patescibacteria phylum (Figure 4.17A) and Paceibacteria class (Figure 4.17B). Accession number for *Minuscule obligatum* MAG: CP174360.

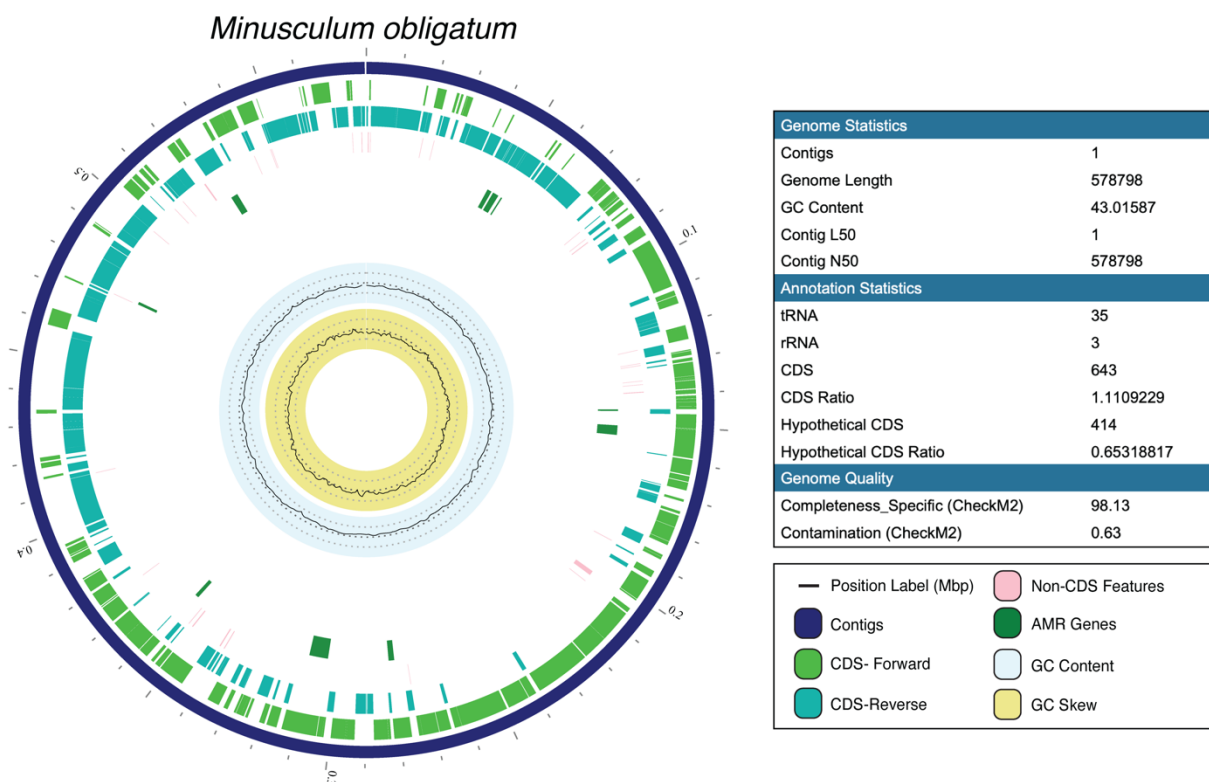


Figure 4.4 Circular genome view of *Minuscolum obligatum*, a MAG recovered from cultures of *N. oceanica* IMET1 and *T. obliquus* HTB1. Genome visualization modified from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC). Genome statistics, annotation statistics, and genome completeness and quality are featured to the right. Figure displayed with permission from Jonas *et al.* (2025b).

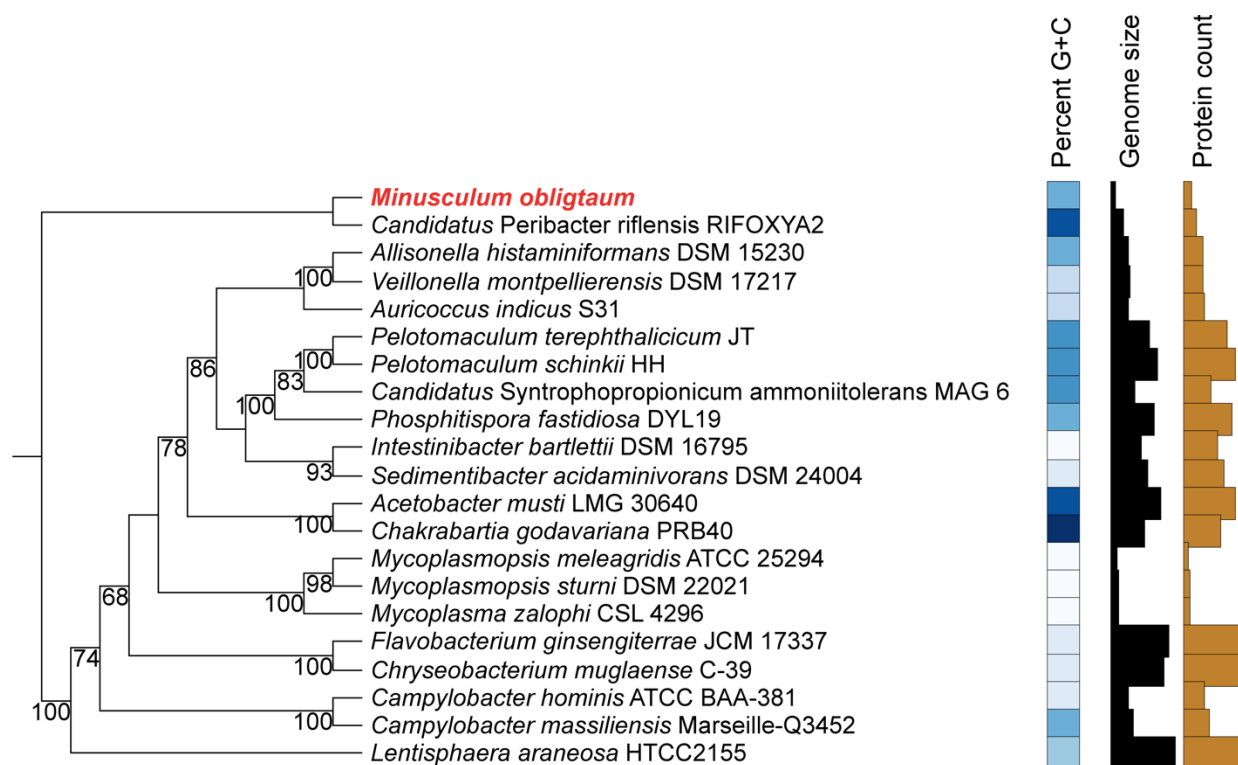


Figure 4.5 Phylogenetic inference of 16S rRNA genes taken from full genome sequences.

Minusculum obligatum was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm. An additional set of ten closely related type strains was determined via the 16S rDNA gene sequences. Distances were calculated using Genome BLAST Distance Phylogeny approach (GBDP).

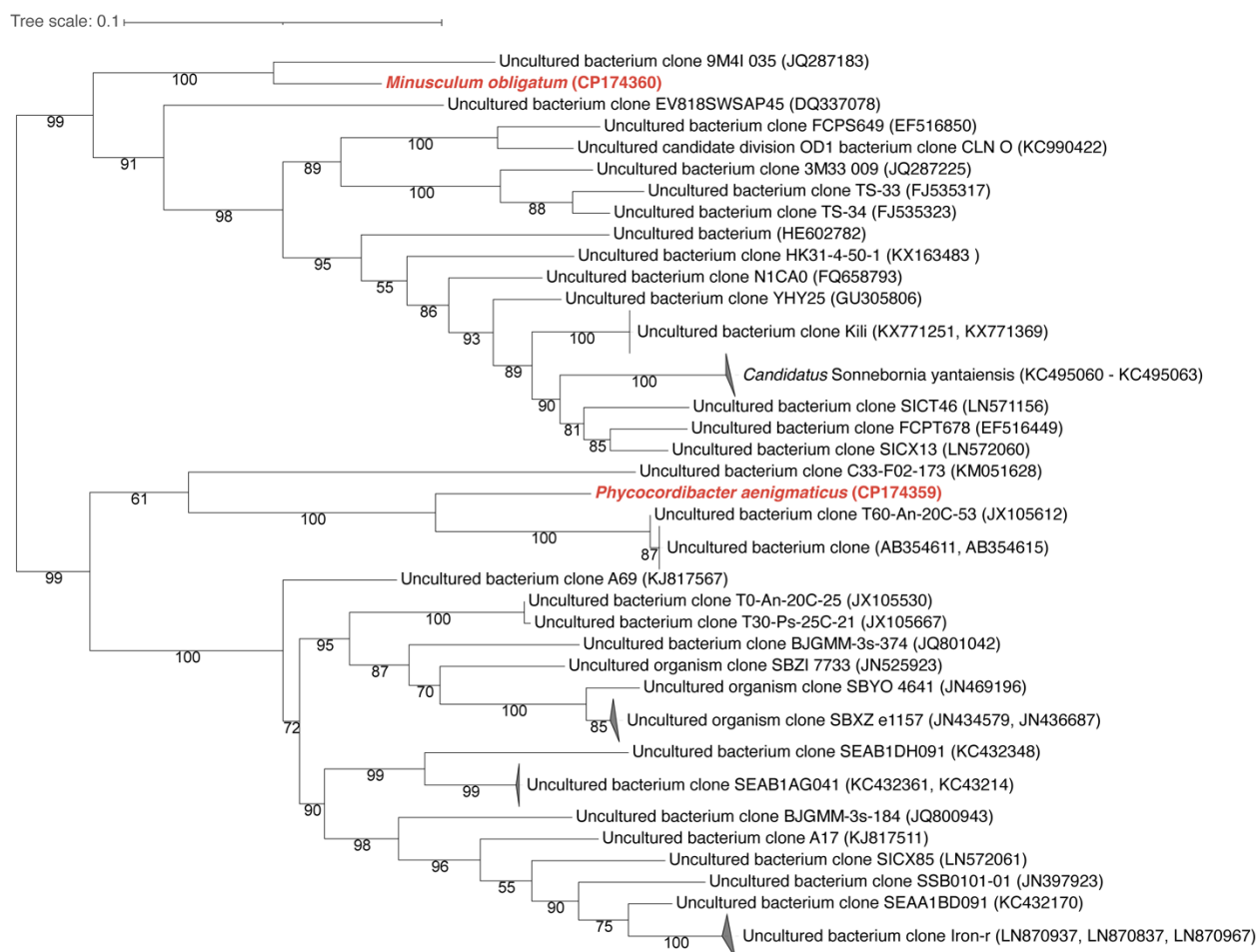


Figure 4.6 Phylogenetic tree deduced from alignment of partial 16S rRNA gene sequences (1,217 bp) of MAGs of this study (red) and nearest hits in the NCBI database. The tree was constructed using the maximum likelihood method and boot strap confidence values (expressed as percentages of 1000 replications) are shown at the branch nodes. Branches with bootstrap values less than 50% were omitted.

3. *Gracilimonas sediminicola*

The genome of *Gracilimonas sediminicola* consists of a single circular chromosome containing 3,629,614 bp and a GC content of 44% (Figure 4.7). The completeness of the genome is 99.87% and the contamination is 0.25% (Table 4.4). In total, 3,328 protein-coding sequences (CDS) were identified, with 1,634 (49%) of the predicted proteins annotated as “hypothetic proteins”. The remaining 51% of sequences were given function assignments, with 642 proteins EC numbers assignments, 550 proteins with GO assignments, 492 proteins with Pathway assignments, 649 proteins with Subsystem assignments, 0 proteins with PLfam assignments, 2230 proteins with PGfam assignments, and 0 proteins with FIGfam assignments. The genome encodes for two large subunit 23S rRNAs (2,068 and 2,912 bp), for one small subunit 16S rRNA (1,530 bp), and for one 5S rRNA (116 bp). The 16S rRNA gene sequence has 99.6% percent identity with those of “*Gracilimonas* sp.” recovered from the Illumina Miseq Platform when the 16S rRNA gene was amplified for prokaryotic barcoding analysis in Chapter 3 (Table 4.5, Figure 3.6 | Chapter 3). The genome encodes 42 tRNAs for all amino acids. Phylogenetic analysis was conducted with deduced from partial alignment of the 16S rRNA gene (1,349 bp) using the NCBI database (Figure 4.8) and taxonomic analysis using GTDB-Tk confirmed the MAG to be *Gracilimonas sediminicola* and can be found at the following accession number: CP175953.

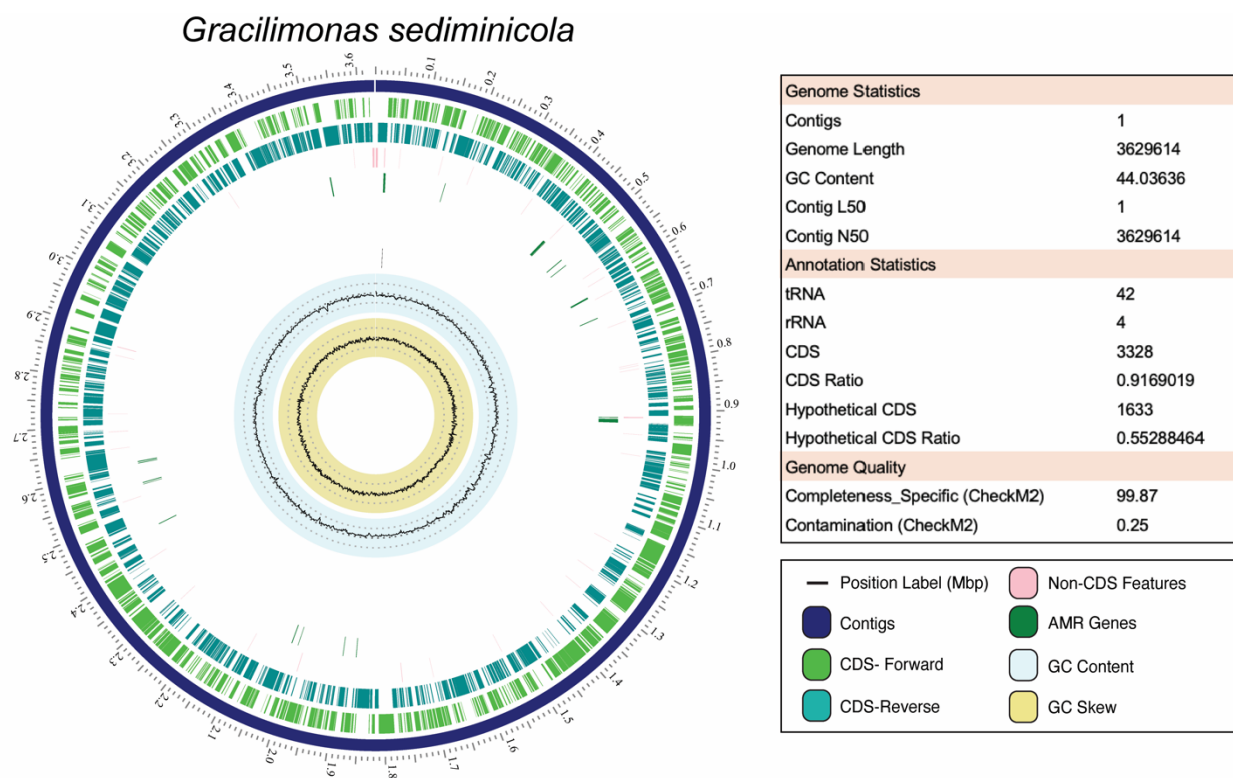


Figure 4.7 Circular genome view of *Gracilimonas sediminicola*, a MAG recovered from cultures of *Nannochloropsis oceanica* IMET1. Visualization modified from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC). Genome statistics, annotation statistics, and genome completeness and quality are shown in the table to the right.

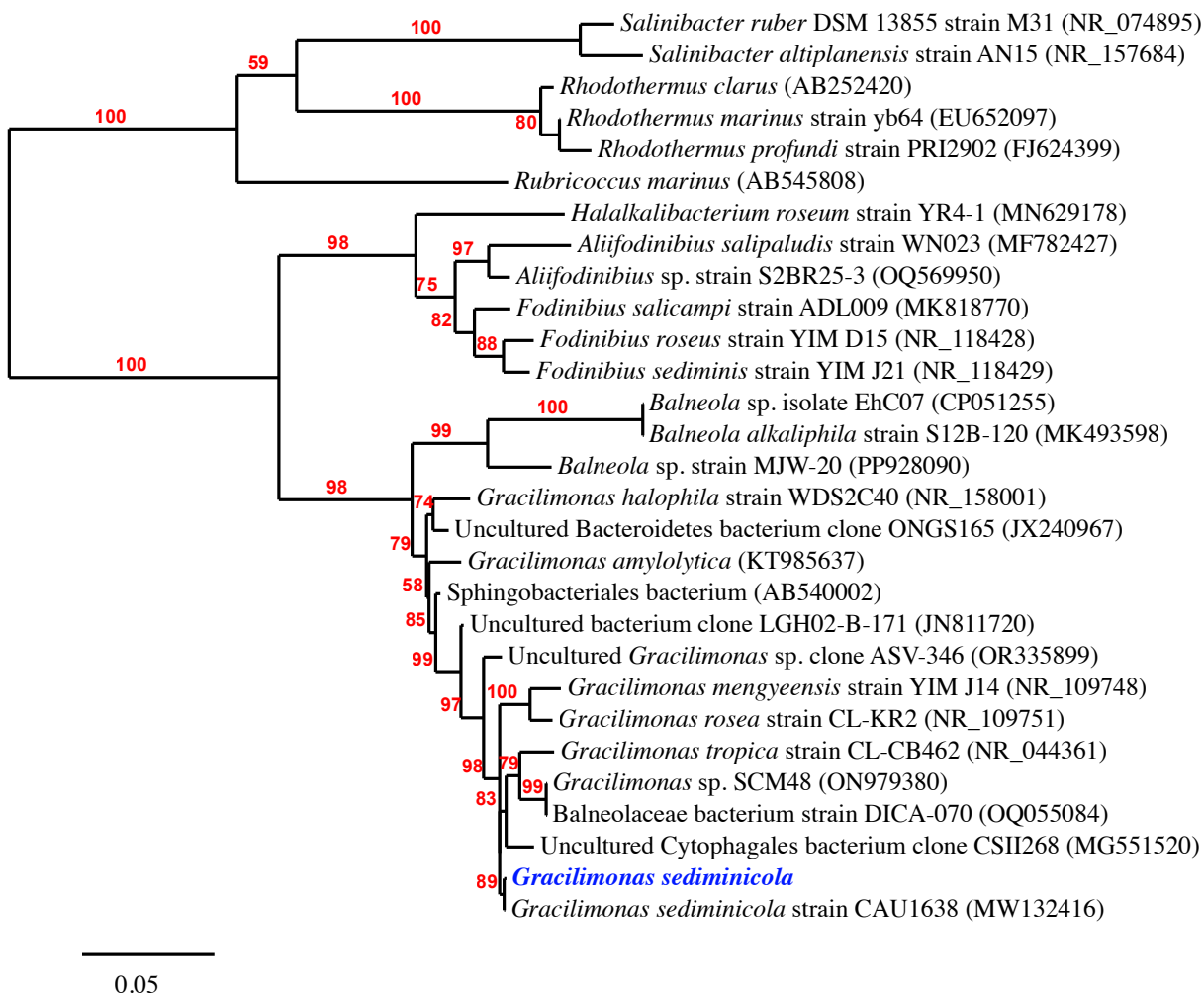


Figure 4.8 Phylogenetic tree deduced from alignment of partial 16S rRNA gene sequences (1,349 bp) of *Gracilimonas sediminicola* MAG of this study (blue) and nearest hits in the NCBI database. The tree was constructed using the maximum likelihood method and boot strap confidence values (expressed as percentages of 1000 replications) are shown at the branch nodes. Branches with bootstrap values less than 50% were omitted.

4. *Microcella pacifica*

The genome of *Microcella pacifica* consists of three contigs containing 2,818,534 bp and a GC content of 69% (Figure 4.9). The completeness of the genome is 81.33% and the contamination is 1.32% (Table 4.4). Figure A4.4 in the Appendix shows that a MAG of *M. pacifica* was recovered in two separate sequencing rounds with an average sequencing depth of 80. In total, 3,078 CDS were identified, with 1,422 (46%) of the predicted proteins annotated as “hypothetic proteins”. The remaining 54% of sequences were given function assignments, with 685 proteins EC numbers assignments, 598 proteins with GO assignments, 523 proteins with Pathway assignments, 678 proteins with Subsystem assignments, 0 proteins with PLfam assignments, 2505 proteins with PGfam assignments, and 0 proteins with FIGfam assignments. The genome encodes for one large subunit 23S rRNAs, one small subunit 16S rRNA (1,529 bp), and for one 5S rRNA (122 bp). The genome encodes 47 tRNAs for all amino acids. The 16S rRNA gene sequence has 99.6% percent identity with those of “*Microcella pacifica*” recovered from the Illumina Miseq Platform when the 16S rRNA gene was amplified for prokaryotic barcoding analysis in Chapter 3 (Table 4.5, Figure 3.8 | Chapter 3). The name was changed to *Microcella pacifica* by NCBI when submitting the genome. Phylogenetic analysis was deduced from full alignment of the 16S rRNA gene using the using the Genome BLAST Distance Phylogeny approach (GBDP) on the Type Strain Genome Server (TYGS) (Figure 4.10). The *Microcella pacifica* MAG can be found at the following accession number: JBJINZ0000000000.

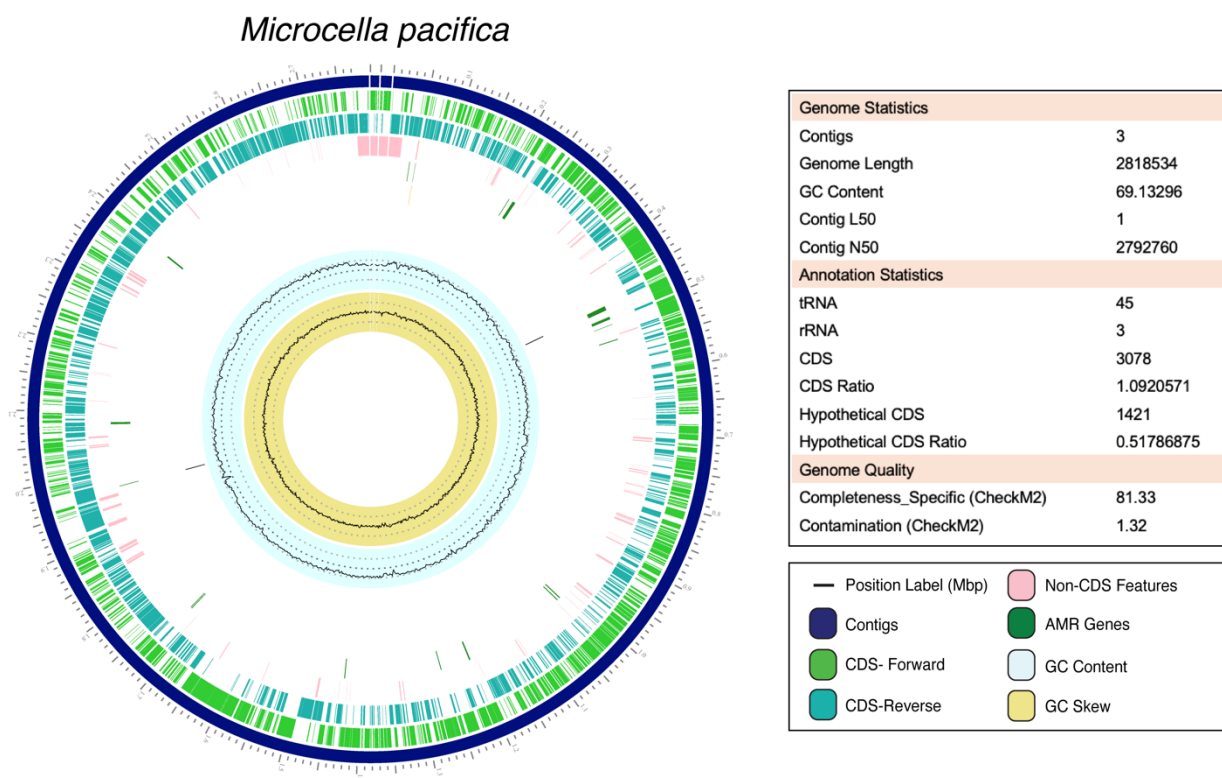


Figure 4.9 Circular genome view of *Microcella pacifica*, a MAG recovered from cultures of *Nannochloropsis oceanica* IMET1. Visualization modified from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC). Genome statistics, annotation statistics, and genome completeness and quality are shown in the table to the right.

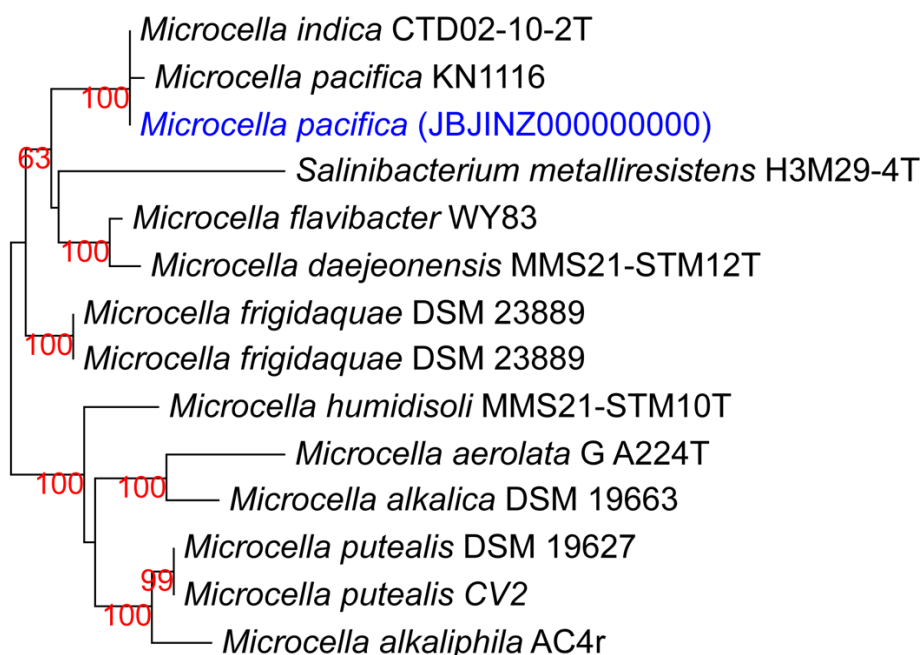


Figure 4.10 Phylogenetic inference of 16S rRNA genes taken from full genome sequences.

Microcella pacifica MAG of this study (blue) was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm. An additional set of ten closely related type strains was determined via the 16S rDNA gene sequences. Distances were calculated using Genome BLAST Distance Phylogeny approach (GBDP).

5 Algoriphagus aqueductus

The genome of *Algoriphagus aqueductus* consists of 94 contigs containing 5,282,570 bp and a GC content of 44.3% (Figure 4.11). The completeness of the genome is 94.45% and the contamination is 5.47% (Table 4.4). In total, 5,213 CDS were identified, with 2,436 (47%) of the predicted proteins annotated as “hypothetic proteins”. The remaining 53% of sequences were given function assignments, with 929 proteins EC numbers assignments, 793 proteins with GO assignments, 707 proteins with Pathway assignments, 885 proteins with Subsystem assignments, 3695 proteins with PLfam assignments, 3848 proteins with PGfam assignments, and 0 proteins

with FIGfam assignments. The genome encodes for three 5S rRNAs, three 23S rRNAs, and three 16S rRNAs (1,529 bp). It also encodes for 52 tRNAs for all amino acids. The 16S rRNA gene sequence has 99.5% percent identity with those of “*Algoriphagus aquaeductus*.” recovered from the Illumina Miseq Platform when the 16S rRNA gene was amplified for prokaryotic barcoding analysis in Chapter 3 (Table 4.5, Figure 3.4 | Chapter 3). Phylogenetic analysis was conducted from full alignment of the 16S rRNA gene using the using the GBDP approach GBDP through TYGS (Figure 4.12). Accession number for *Algoriphagus aquaeductus* MAG: JBJINY0000000000.

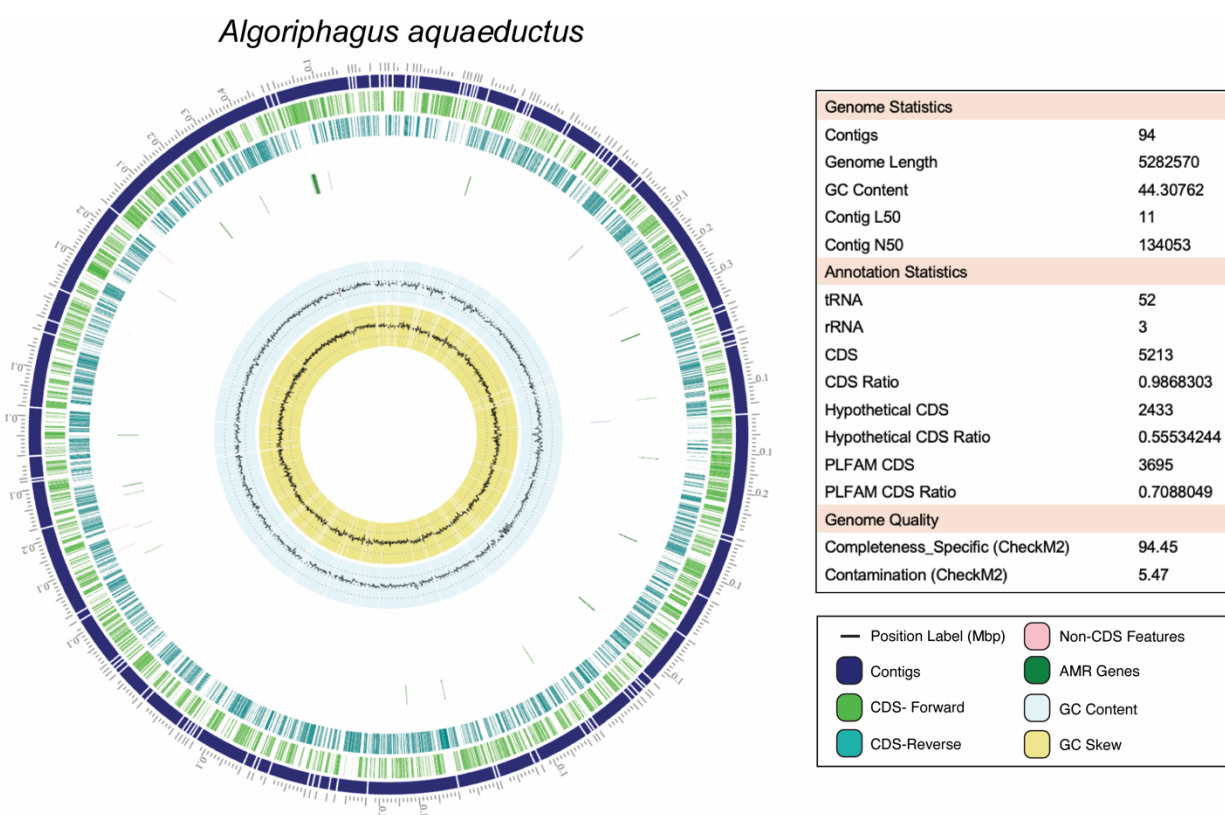


Figure 4.11 Circular genome view of *Algoriphagus aquaeductus*, a MAG recovered from cultures of *Tetrademus obliquus* HTB1. Visualization modified from the Bacterial and Viral

Bioinformatics Resource Center (BV-BRC). Genome statistics, annotation statistics, and genome completeness and quality are shown in the table to the right.

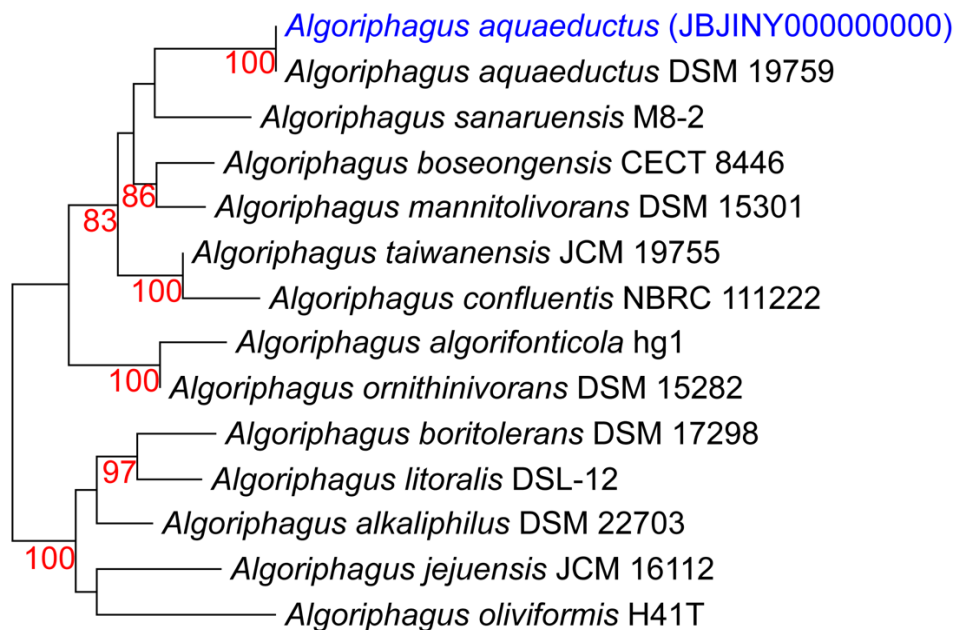


Figure 4.12 Phylogenetic inference of 16S rRNA genes taken from full genome sequences. *Algoriphagus aquaeductus* MAG of this study (blue) was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm. An additional set of ten closely related type strains was determined via the 16S rDNA gene sequences. Distances were calculated using Genome BLAST Distance Phylogeny approach (GBDP).

6. *Erythrobacter* sp.

The genome of *Erythrobacter* sp. consists of 56 contigs containing 3,211,232 bp and a GC content of 63.6% (Figure 4.13). The completeness of the genome is 99.31% and the contamination is 1.64% (Table 4.4). In total, 3,218 protein-coding sequences (CDS) were

identified, with 1,197 (37%) of the predicted proteins annotated as “hypothetic proteins”. The remaining 63% of sequences were given function assignments, with 788 proteins EC numbers assignments, 664 proteins with GO assignments, 623 proteins with Pathway assignments, 855 proteins with Subsystem assignments, 0 proteins with PLfam assignments, 2549 proteins with PGfam assignments, and 0 proteins with FIGfam assignments. The genome encodes for one large subunit 23S rRNA, for one small subunit 16S rRNA (1,495 bp), and for one 5S rRNA. The 16S rRNA gene sequence has 99.5% percent identity with those of “*Erythrobacter* sp.” recovered from the Illumina Miseq Platform when the 16S rRNA gene was amplified for prokaryotic barcoding analysis in Chapter 3 (Table 4.5, Figure 3.4 | Chapter 3). The genome also encodes 45 tRNAs for all amino acids. Phylogenetic analysis was conducted from full alignment of the 16S rRNA gene using the using the GBDP approach GBDP through TYGS (Figure 4.14). Accession number for *Erythrobacter* sp. MAG: JBJIOB0000000000.

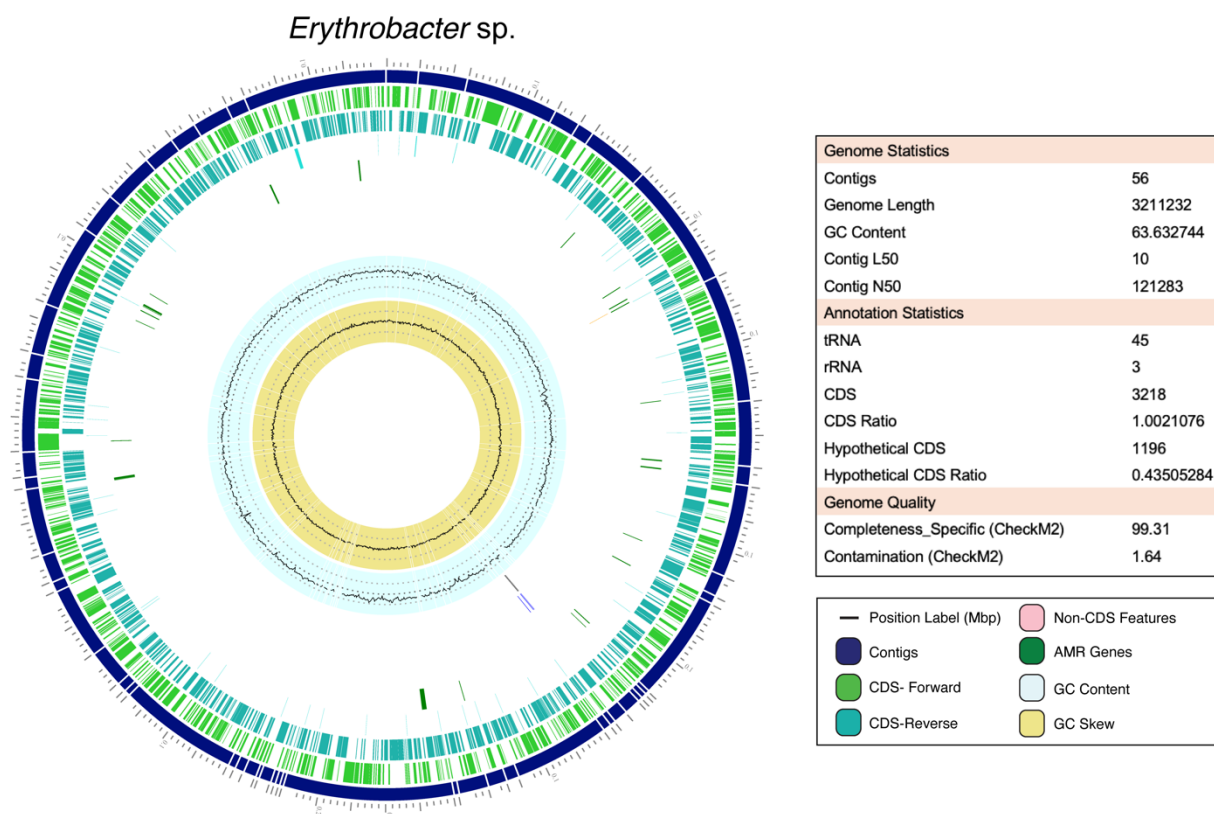


Figure 4.13 Circular genome view of *Erythrobacter* sp., a MAG recovered from cultures of *Tetrademus obliquus* HTB1. Visualization modified from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC). Genome statistics, annotation statistics, and genome completeness and quality are shown in the table to the right.

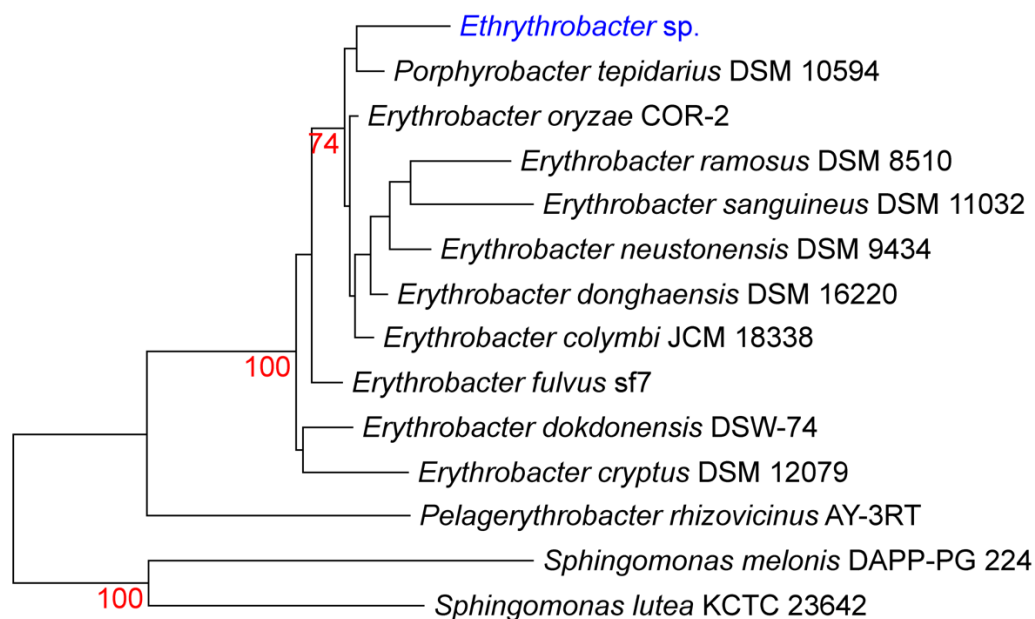


Figure 4.14 Phylogenetic inference of 16S rRNA genes taken from full genome sequences *Erythrobacter* sp. MAG of this study (blue) was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm. An additional set of ten closely related type strains was determined via the 16S rDNA gene sequences. Distances were calculated using Genome BLAST Distance Phylogeny approach (GBDP).

7. Allorhizobium sp.

The genome of *Allorhizobium* sp. consists of 57 contigs containing 5,109,754 bp and a GC content of 60.2% (Figure 4.15). The completeness of the genome is 95.74% and the contamination is 2.09% (Table 4.4). In total, 5,255 protein-coding sequences (CDS) were identified, with 1,800 (34%) of the predicted proteins annotated as “hypothetic proteins”. The remaining 66% of sequences were given function assignments, with 1128 proteins EC numbers assignments, 964 proteins with GO assignments, 869 proteins with Pathway assignments, 1343 proteins with Subsystem assignments, 0 proteins with PLfam assignments, 4626 proteins with

PGfam assignments, and 0 proteins with FIGfam assignments. The genome encodes for two large subunit ribosomal 23S rRNAs, for two small subunit 16S rRNAs (1,493 bp), and for two 5S rRNAs. The 16S rRNA gene sequence has 99.76% percent identity with those of “*Allorhizobium* sp.” recovered from the Illumina Miseq Platform when the 16S rRNA gene was amplified for prokaryotic barcoding analysis in Chapter 3 (Table 4.5, Figure 3.4 | Chapter 3). The genome also encodes 52 tRNAs for all amino acids. Phylogenetic analysis was conducted from full alignment of the 16S rRNA gene using the using the GBDP approach GBDP through TYGS (Figure 4.16). The classification of *Allorhizobium* sp. was provided by the GTDB and MiGA. Accession number for *Allorhizobium* sp. MAG: JBJIOA000000000

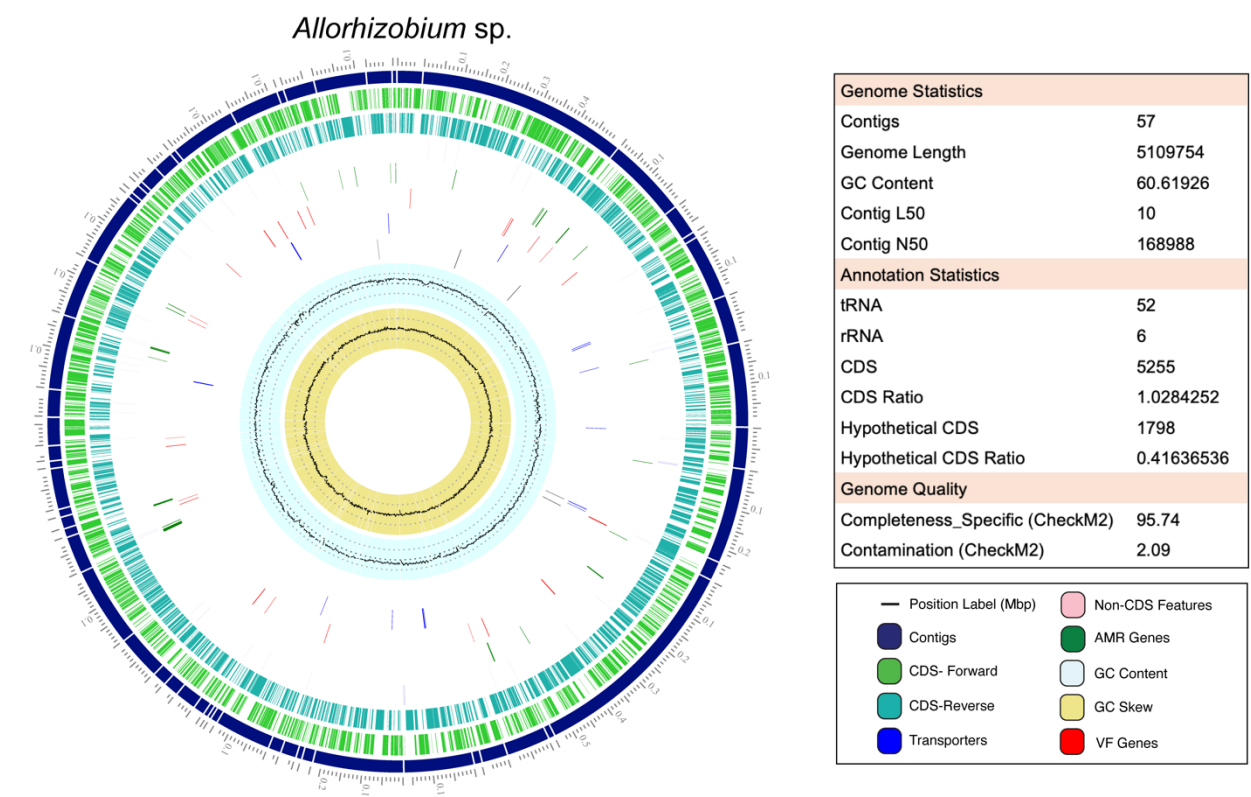


Figure 4.15 Circular genome view of *Allorhizobium* sp., a MAG recovered from cultures of *Tetrademus obliquus* HTB1. Visualization modified from the Bacterial and Viral

Bioinformatics Resource Center (BV-BRC). Genome statistics, annotation statistics, and genome completeness and quality are shown in the table to the right.

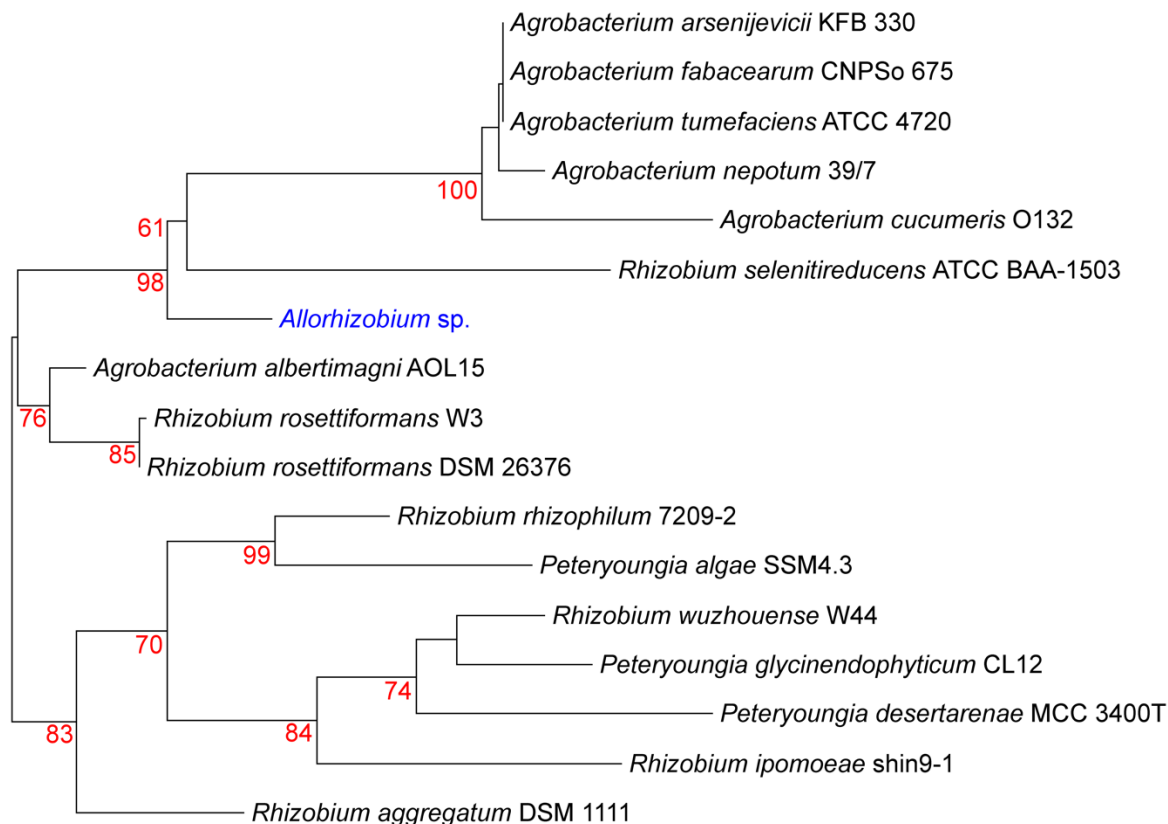


Figure 4.16 Phylogenetic inference of 16S rRNA genes taken from full genome sequences *Allorhizobium* sp. MAG of this study (blue) was compared against all type strain genomes available in the Type Strain Genome Database (TYGS) via the MASH algorithm. An additional set of ten closely related type strains was determined via the 16S rDNA gene sequences. Distances were calculated using Genome BLAST Distance Phylogeny approach (GBDP).

Table 4.4 Genome characteristics of the MAGs of this study. Completeness and contamination estimates have been calculated with CheckM2 and the Completeness_specific parameter, which applies a lineage-specific set of single-copy marker genes tailored to the genome's taxonomic group. Abbreviations: bp = base pairs, CDS = coding DNA sequences, N50 = the length of the shortest contig (in bp) at which half of the entire genome assembly is contained in contigs of this length or longer.

	<i>Minusculeum obligatum</i>	<i>Phycocordibacter aenigmaticus</i>	<i>Gracilimonas sediminicola</i>	<i>Microcella pacific</i>	<i>Algoriphagus aqueductus</i>	<i>Erythrobacter sp.</i>	<i>Allorhizobium sp.</i>
Accession #	CP174360	CP174359	CP175953	JBJNZ0000 00000	JBJNY0000 00000	JBJOB00000 0000	JBJOA000000 000
Genome size (bp)	578,798	721,362	3,629,614	2,818,534	5,282,570	3,211,232	5,109,754
Contig count	1	1	1	3	94	56	57
Completeness (%)	98.13	99.99	99.87	81.33	94.45	99.31	95.74
Contamination (%)	0.63	0.54	0.25	1.32	5.47	1.64	2.09
GC content (%)	43.02	44.05	44.04	69.13	44.31	63.63	60.62
N50	578,798	721,362	3,629,614	2,792,760	134,053	121,283	168,988
CDS	645	768	3,328	3,078	5,213	3,218	5,255
L50	1	1	1	1	11	10	10
Ribosomal RNAs	5S (70%), 16S, 23S	5S (55%), 16S, 23S	5S, 16S, 23S	5S, 16S, 23S	5S, 16S, 23S	5S, 16S, 23S	5S, 16S, 23S
tRNA count	35	36	42	47	52	45	52

Table 4.5 Comparison of the 16S rRNA gene sequence recovered from the same DNA extract sequenced with different platforms

Species	16S rRNA gene sequence amplified using Illumina MiSeq (Chapter 3)	16S rRNA gene sequence from metagenomic sequencing (Nanopore)	Query cover (%)	% identity
<i>Minusculture obligatum</i>	444 bp	1,446 bp	100	99.55
<i>Phycocordibacter aenigmaticus</i>	444 bp	1,465 bp	100	99.55
<i>Gracilimonas sediminicola</i>	460 bp	1,530 bp	100	99.57
<i>Microcella pacifica</i>	445 bp	1,529 bp	100	99.55
<i>Algoriphagus aqueductus</i>	439 bp	1,529 bp	100	100
<i>Erythrobacter</i> sp.	420 bp	1429 bp	100	99.52
<i>Allorhizobium</i> sp.	420 bp	1493 bp	100	99.76

4.4.3 Establishing taxonomic novelty of two MAGs

Three of the seven MAGs presented here have been taxonomically classified to the species-level, *Gracilimonas sediminicola*, *Microcella pacifica*, and *Algoriphagus aqueductus*. Two have been taxonomically classified to the genus-level, *Erythrobacter* sp. and *Allorhizobium* sp. When originally analyzed during the course of this research, *Minusculture obligatum* and *Phycocordibacter aenigmaticus* were only given the rank of class (Patescibacteria) with no further taxonomic resolution. Thus, the remaining work of this chapter aimed to classify these two Patescibacteria by establishing taxonomic novelty and generating nomenclature, both of which were validated by the Seqcode.

To establish taxonomic novelty, overall genome related indices (OGRIs), namely ANI and dDDH were not sufficient as they are typically used to delineate species clusters as opposed to higher ranks (Parks *et al.*, 2022). In addition to OGRIs, we relied on AAI, multigene-based phylogenies (bac120), and RED scores using a combination of software tools to provide a robust scaffold for microbial taxonomic delineation. The metrics comparing *P. aenigmaticus* to its nearest neighbor in multiple databases (MiGA TypeMat, NCBI, and GTDB)—*i.e.* an AAI of 37.03% and a RED score of 0.92162 (Table 4.7)—in combination with no classified neighbors in 16S rRNA phylogenetic analysis (Figure 4.6) and MPB phylogenetic analysis (Figure 4.17) led to the proposal of the novel genus *Phycocordibacter* gen. nov. and higher ranks. Similar results for *M. obligatum* (AAI of 36.75% and RED score of 0.93255) lead to the proposal of the novel genus *Minusculum* gen. nov.

4.4.4 Formal proposal of *Minusculum obligatum* gen. nov. sp. nov., *Phycocordibacter aenigmaticus* gen. nov. sp. nov., and associated higher taxonomic ranks

I formally proposed the following taxonomic names for two novel genera, two novel species, one novel family, and one novel order: *Minusculum obligatum* gen. nov. sp. nov., *Phycocordibacter aenigmaticus* gen. nov. sp. nov., Phycocordibacteraceae fam. nov., and Phycocordibacterales ord. nov. (Table 4.6) (Jonas *et al.*, 2025b [in review]). The bac120 tree classified the two MAGs as having belonged to the same phylum (Patescibacteria), same class (Paceibacteria), same order (Phycocordibacterales, replacing the order placeholder o__UBA9973), same family (Phycocordibacteraceae, replacing the family placeholder f__UBA2103), two distinct genera (*Phycocordibacter*, replacing the genus placeholder g__UBA2103; *Minusculum*, replacing the genus placeholder g__1-14-0-10-45-20) (Figure 4.17C, Table 4.6). Data from MiGA reflecting taxonomic novelty (AAI %), digital DNA

hybridization (dDDH) values provided by the TYGS, and RED scores using GTDB-Tk support this proposal of novelty that has been validated by the Seqcode, (seqco.de/r:ywe1blo2) (Riesco and Trujillo, 2024) (Table 4.7). The closest relatives found by MiGA for *P. aenigmaticus* in the TypeMat database was *Ammonifex degensii* KC4 (37.03% AAI) and *Pueribacillus theae* GCF (36.75% AAI) for *M. obligatum* (Table 4.7). TYGS assigned *Chlamydia psittaci* 6BC as the closest subject strain to *P. aenigmaticus* with a highest dDDH of 13.2% (Table 4.7, Figure 4.3) and *Ca. Peribacter riflensis* to *M. obligatum* with a dDDH of 16.9% (Table 4.7, Figure 4.5). RED scores using the bac120 alignment (Figure 4.17) were 0.92162 for *P. aenigmaticus* and 0.93255 for *M. obligatum*.

4.4.5 Description of *Minuscolum obligatum*:

I proposed *Minuscolum obligatum* gen. nov. sp. nov. to replace the alphanumeric genus place holder g__1-14-0-10-45-20 (Jonas *et al.*, 2025b [in review]). Species etymology: *M. obligatum*—syllabification o.bli.ga'tum—from Latin neut. adj. *obligatum*, to bind, oblige, or require. Genus etymology: *Minuscolum*—Mi.nu.scu.lum—Latin neut. noun, meaning rather small, referring to the genome size. Thus, *Minuscolum obligatum*, an obligate symbiont with a highly reduced genome (~600K bp). A formal protologue has been created for this proposed name in Table 4.7 (Chuvochina *et al.*, 2019).

4.4.6 Description of *Phycocordibacter aenigmaticus*:

I proposed *Phycocordibacter aenigmaticus* gen. nov. sp. nov. to replace the alphanumeric genus place holder g__UBA2103 (Jonas *et al.*, 2025b [in review]). Species etymology: *P. aenigmaticus*—syllabification ae.nig.ma'ti.cus—from Latin masculine adjective *aenigmaticus*, meaning enigmatic or obscure (Table 4.7). Genus etymology: *Phycocordibacter*—

Phy.co.cor.di.bacter—is split into three distinct morphemes: Greek neuter noun phyco, from *phykos*, meaning seaweed or algae; L. neut. n. cor, from *cordis*, meaning central or essential; N.L. masc. n. bacter, referring to a bacterium. Thus, N.L. masc. n. *Phycocordibacter aenigmaticus*, an enigmatic and core bacteria of a microalgal culture. The inferred stem, Phycocordibacter-, was used to create subsequent family and order level nomenclature.

4.4.7 Description of Phycocordibacteraceae fam. nov.

I proposed Phycocordibacteraceae (Phy.co.cor.di.bac.ter.a'ce.ae. Latin masc. noun) to replace family placeholder f__UBA2103 (Jonas *et al.*, 2025b [in review]). Phycocordibacter is the Candidatus type genus of the family; -aceae ending to denote a family. Thus, N.L. fem. pl. n. Phycocordibacteraceae, the Phycocordibacter family. *Minusculum obligatum* also falls within this proposed family.

4.4.8 Description of Phycocordibacterales ord. nov.

I proposed Phycocordibacterales (Phy.co.cor.di.bac.ter.ales. Latin masc. noun) to replace order placeholder o__UBA9973 (Jonas *et al.*, 2025b [in review]). Phycocordibacter is the Candidatus type genus of the order; -ales ending to denote an order. Thus, N.L. fem. pl. n. Phycocordibacterales, the Phycocordibacter order. *Minusculum obligatum* also falls within this proposed order.

Table 4.6. List of proposed names and their current placeholders.

Proposed type MAG accession	Proposed species name	Previous name of order	Proposed order name	Previous name of family	Proposed family name	Previous name of genus	Proposed genus name
CP174359	<i>Phycocordibacter aenigmaticus</i>	o__UBA9973	Phycocordibacter ales	f__UBA2103	Phycocordibacter aceae	g__1-14-0-10-45-20	<i>Phycocordibacter</i>
CP174360	<i>Minuscolum obligatum</i>	o__UBA9973	Phycocordibacter ales	f__UBA2103	Phycocordibacter aceae	g__UBA2103	<i>Minuscolum</i>

Table 4.7. Protologues of the MAGs of this study as suggested by Chuvochina et al., 2019 and the proposed minimal standards set forth by Riesco and Trujillo, 2024. ANI= Average Nucleotide Identity. AAI = Average Amino Acid Identity. dDDH = digital DNA hybridization. RED = Relative Evolutionary Divergence. Symbol '--' denotes non-applicability with certain rows regarding taxonomic novelty. * = AAI % of closest relatives found by MiGA using the TypeMat database. ** = dDDH formula d₄ recommended by the TYGS. ‡ = Taxonomic novelty determined by MiGA using the TypeMat database.

	<i>Minuscolum obligatum</i>	<i>Phycocordibacter aenigmaticus</i>	<i>Gracilimonas sediminicola</i>	<i>Microcella pacifica</i>	<i>Algoriphagus aquaeductus</i>	<i>Erythrobacter sp.</i>	<i>Allorhizobium sp.</i>
Accession #	CP174360	CP174359	CP175953	JBJINZ000000000	JBJINY000000000	JBJIOB000000000	JBJIOA000000000
Species name	<i>Minuscolum obligatum</i>	<i>Phycocordibacter aenigmaticus</i>	--	--	--	--	--
Genus name	<i>Minuscolum</i>	<i>Phycocordibacter</i>	--	--	--	--	--
Specific epithet	<i>obligatum</i>	<i>aenigmaticus</i>	--	--	--	--	--

Genus etymology	L. neut. n. Minusculum, rather small, referring to the small genome size	Gr. neut. n. phyco, meaning seaweed or algae; L. neut. n. cor, from <i>cordis</i> , meaning central or essential; N.L. masc. n. bacter, referring to a bacterium; N.L. masc. n. Phycocordibacter, a core bacteria of a microalgal culture		--	--	--	--
Species etymology	L. neut. adj. obligatum, to bind, oblige, or require	L. masc. adj. aenigmaticus, enigmatic or obscure	--	--	--	--	--
Genus status	gen. nov.	gen. nov.	--	--	--	--	--
Species status	sp. nov.	sp. nov.	--	--	--	--	--
Pairwise ANI (%)	N/A	N/A	99.48	97.71	98.06	N/A	N/A
AAI (%)*	36.75%	37.03%	--	--	--	--	--
dDDH (%) **	16.9%	13.2%	--	--	--	--	--
RED score	0.93255	0.92162					
Taxonomic novelty (p-value ≤ 0.01 suggests novelty) ‡	Phylum= 0.123 Class= 0.00622 Order= 0.000576 Family= 0.00025 Genus= 0.000246	Phylum= 0.143 Class= 0.0101 Order= 0.00133 Family= 0.000379 Genus= 0.000246	--	--	--	--	--

Designation of the type MAG	CP174360	CP174359	GCA_024320 785.1	GCF_0093719 75.2	GCF_0032534 85.1	No type designated	GCF_01331801 5.2
Status of MAG	Draft	Draft	Draft	Draft	Draft	Draft	Draft
Genome size (bp)	578,798	721,362	3,629,614	2,818,534	5,282,570	3,211,232	5,109,754
GC content (%)	43.02	44.05	44.04	69.13	44.31	63.63	60.62
Completeness (%)	98.13	99.99	99.87	81.33	94.45	99.31	95.74
Contamination (%)	0.63	0.54	0.25	1.32	5.47	1.64	2.09
Contig Count	1	1	1	3	94	56	57
N50	578,798	721,362	3,629,614	2,792,760	134,053	121,283	168,988
L50	1	1	1	1	11	10	10
Coverage	5,609	380	140	85	145	16	15
Country of origin	USA	USA	USA	USA	USA	USA	USA
Region of origin	Baltimore, Maryland	Baltimore, Maryland	Baltimore, Maryland	Baltimore, Maryland	Baltimore, Maryland	Baltimore, Maryland	Baltimore, Maryland
Source of sample	Microalgal culture	Microalgal culture	Microalgal culture	Microalgal culture	Microalgal culture	Microalgal culture	Microalgal culture
Algal host name	<i>N. oceanica</i> & <i>T. obliquus</i>	<i>N. oceanica</i> & <i>T. obliquus</i>	<i>N. oceanica</i>	<i>N. oceanica</i>	<i>T. obliquus</i>	<i>T. obliquus</i>	<i>T. obliquus</i>
Sampling date	2022-05-01	2023-01-20	2022-05-01	2022-05-01	2021-12-12	2021-12-12	2021-12-12
Geographic location	Institute of Marine and Environmental Technology	HyTek Bio LLC	Institute of Marine and Environmental Technology	Institute of Marine and Environmental Technology	Institute of Marine and Environmental Technology	Institute of Marine and Environmental Technology	Institute of Marine and Environmental Technology
Latitude	39.2860027 N	39.298342 N	39.2860027 N	39.2860027 N	39.2860027 N	39.2860027 N	39.2860027 N

Longitude	76.6056885 W	76.496257 W	76.6056885 W	76.6056885 W	76.6056885 W	76.6056885 W	76.6056885 W
Assembly	3 samples	3 samples	1 sample	1 sample	1 sample	1 sample	1 sample
Sequencing platform	Oxford Nanopore GridION	Oxford Nanopore GridION	Oxford Nanopore GridION	Oxford Nanopore GridION	Oxford Nanopore GridION	Oxford Nanopore GridION	Oxford Nanopore GridION
Assembly program	Flye v2.9.2	Flye v2.9.2	Flye v2.9.2	Flye v2.9.2	Flye v2.9.2	Flye v2.9.2	Flye v2.9.2
Binning program	MaxBin2 v2.2.4	MaxBin2 v2.2.4	MaxBin2 v2.2.4	MaxBin2 v2.2.4	CONCOCT v1.1	CONCOCT v1.1	CONCOCT v1.1
Predicted proteins	641	749	3,243	3,009	4,770	3,143	5,052
Ribosomal RNAs	5S (70%), 16S, 23S	5S (55%), 16S, 23S	5S, 16S, 23S	5S, 16S, 23S	5S, 16S, 23S	5S, 16S, 23S	5S, 16S, 23S
tRNA count	35	36	42	47	52	45	52
BioSample	SAMN44533063	SAMN44533074	SAMN44533075	SAMN44533076	SAMN44533081	SAMN44533102	SAMN44533082

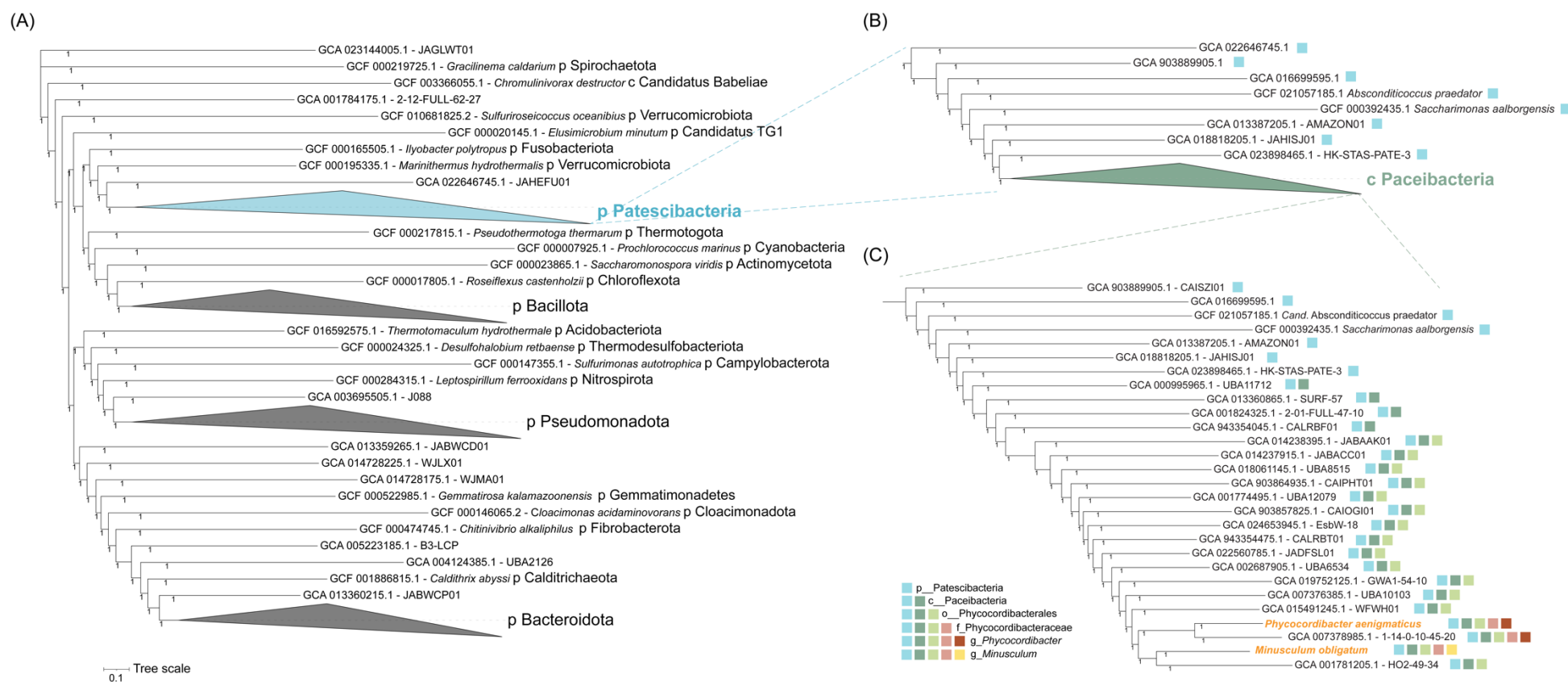


Figure 4.17 Phylogenetic tree visualizing alignment of 120 concatenated universal bacterial genes generated by GTDB-Tk at the (A) phylum level, (B) class level, and (C) subsequent taxonomic ranks. Bootstrap support values are shown at branch nodes and multiple members of strongly supported, monophyletic bacterial phyla were collapsed and shown by triangle using iTOL. Light blue shapes denote members of the Patescibacteria phylum and dark green shapes denote members of the Paceibacteria class. Light green squares denote members belonging to the order Phycocordibacterales ord. nov. Pink squares denote members belonging to the family

Phycocordibacteraceae fam. nov. Red squares denote two members belonging to the genus *Phycocordibacter* and yellow square denotes one member of the genus *Minusculum*. The collapsed blue triangle in (A) is the tree in (B). Further, the collapsed green triangle in (B) is the tree in (C). Figure displayed with permission from Jonas *et al.* (2025b).

4.5 Discussion

4.5.1 Assembly of seven high quality MAGs from bacteria associated with two microalgal cultures

The Bacterial and Viral Bioinformatics Resource Center (BV-BRC) combines various tools to determine a genome quality score. First, EvalG checks the completeness and contamination of the genome by identifying universal genes that are anticipated to occur only once in genomes of a particular taxonomic grouping (Parks *et al.*, 2015; Parrello *et al.*, 2019). Missing genes thus indicate the genome is less complete, and extra genes indicate the genome may be contaminated. Second, EvalCon checks the coarse and fine consistency of the genome annotation, which is calculated by determining how many instances of each gene are expected (Parrello *et al.*, 2019). When a gene is unexpected, or an expected gene is missing, this is defined as coarse inconsistency. If a gene occurs a different number of times than predicted, this is defined as fine inconsistency. The genome quality score considers these four metrics: completeness, contamination, coarse consistency, and fine consistency. The genome quality for all seven MAGs of this study were considered “Good” by the BV-BRC. This finding was further bolstered according to the Minimum Information about a Metagenome-Assembled Genome (MIMAG) criteria, which defines high quality MAGs with the following metrics: Completion >90%; Contamination <5%; Presence of the 23S, 16S, and 5S rRNA genes and at least 18 tRNAs (47).

Two MAGs barely fell short of these metrics, *Algoriphagus aquaeductus* with a contamination of 5.47% and *Microcella pacifica* with a completeness of 81.33%. The lower completeness score for *M. pacifica* was surprising because the MAG was recovered from two

distinct sequencing rounds, one in June 2023 and the other in April 2024. The largest contig for each MAG recovered was ~2.8 Mb and had a coverage of ~80 (Appendix, Figure A4.4). This indicates the low completeness score was likely not from insufficient sequencing depth. Further, the type genome for *M. pacifica* provided by NCBI (GCF_009371975.2) is 2.7 Mb in length. Thus, I hypothesize there may be features within the *M. pacifica* genome, such as the presence of repetitive regions that resulted in lower completeness scores when using CheckM and CheckM2. It could also be the case that *M. pacifica* has a linear chromosome, similar to other Actinomycetes. For example, linear genomes of various *Streptomyces* spp. are usually capped with terminal inverted repeats, which can present challenges in genome assembly (Jørgensen *et al.*, 2024). Therefore, I kept *M. pacifica* in my dataset for further analysis despite its completeness score of 81.33%.

It is common for studies involving metagenome assembly to retain any MAG with a completeness score of 50% or higher (Chang *et al.*, 2024; Feng and Li, 2024). Although hundreds of metagenomes can be recovered this way, these incomplete assemblies can often encumber already overloaded databases and any inference taken from the genome needs to be carefully scrutinized. Instead, the intention for my dissertation was to glean information from only reads that had a high sequencing depth, meaning the same sequence showed up >50 times in the sequencing run. This allows for the annotation of high-quality MAGs and minimizes incorrect extrapolation from corrupt sequences.

4.5.2 Unexpected and unprecedented dominance of novel Patescibacteria within microalgal cultures

Patescibacteria have rarely been reported to be associated with microalgae apart from microbial fuel cells with algal-film cathodes (Wang *et al.*, 2024) and algal-bacterial biofilms in wastewater treatment facilities (Wang *et al.*, 2022; Chang *et al.*, 2023; Sun *et al.*, 2024). The latter is to be expected as Patescibacteria are commonly found within wastewater treatment plants (Hu *et al.*, 2024). For example, a Patescibacteria (order Saccharimonadales) emerged as the dominant taxon in activated sludge that was aerated by a separated microalgal biofilm reactor. Although this bacterium was not in contact with the microalgae (*T. obliquus*), the bacterial community clearly shifted after 10 days with Patescibacteria becoming the most abundant phylum at 44% relative abundance (Huang *et al.*, 2024). It is likely that other varied members of Patescibacteria have been found within algal microbiomes, although at a lower relative abundance and not taxonomically classified. For example, there have been reports of an “uncultured marine bacterium” with phylogenetic affiliations to *Cand.* Gracilibacteria associated with marine macroalgae and an “uncultured bacterium” with phylogenetic affiliations to *Cand.* Peregrinibacteria associated with diatom blooms (Rahlff *et al.*, 2020).

Although it is common practice to find uncultivated bacteria within microbiome data (Lloyd *et al.*, 2018), it was unexpected that two ‘uncultured bacterium clones’ were found in high relative abundance repeatedly throughout multiple experiments and at multiple scales, both within this study: 500 L bioreactor (Figure 3.10 | Chapter 3), and past studies conducted by our laboratory: 1 L scale (Lee *et al.*, 2024), 1 L and 500 L bioreactors (Jonas *et al.*, 2025a). Neither bacterium was found within *T. obliquus* cultures at the 1 L scale (Figure 3.4 | Chapter 3). Before metagenomic sequencing was conducted, the 16S rRNA genes of the two Patescibacteria were

thought to have come from one organism (Lee *et al.*, 2024). I have since established that two distinct and novel members of the Paceibacteria class were found and now serve as type strains for two novel genera, one novel family, and one novel order.

4.5.3 Genome reduction and a lack of essential genes: familiar features of Patescibacteria

Through 16S rRNA gene analysis, Patescibacteria have been found to represent approximately 50% of bacterial lineages of phylum-level evolutionary distance (Hug *et al.*, 2016). However, this could be due to atypical evolution of ribosomal proteins as Parks *et al.* (2017) reported Patescibacteria comprise approx. 25% of phylum-level lineages when phylogeny was constructed based on 120 concatenated proteins (Parks *et al.*, 2017); others have reported lower than 25% (Schulz *et al.*, 2017). Two seemingly constant features of Patescibacteria are small genomes and ultra-small cell sizes (Castelle *et al.*, 2018; Gios *et al.*, 2023). For example, several Patescibacteria were reported in groundwater samples that passed through 0.22 μm filters with cells sizes of $0.009 \pm 0.002 \mu\text{m}^3$ (Luef *et al.*, 2015). Genome sizes are most commonly reported to be <1 Mb with this study being no exception, reporting *M. obligatum* at 578,798 bp (Figure 4.4) and *P. aenigmaticus* at 721,362 bp (Figure 4.2). Reduced genome sizes lead to another hallmark feature of Patescibacteria, limited metabolic capabilities which will be the focus of Chapter 5 (Rinke *et al.*, 2013; Brown *et al.*, 2015; Castelle *et al.*, 2018).

Chapter 5. Metabolic pathway reconstruction and functional gene analysis of bacteria associated with *Tetradismus obliquus* HTB1 and *Nannochloropsis oceanica* IMET1

5.1 Abstract

The functional roles of bacterial symbionts associated with microalgae remain understudied despite the importance of microalgae in biotechnology and environmental microbiology. I used METABOLIC, GapMind, and R to conduct functional gene analysis and metabolic reconstruction on the metagenomic sequence datasets described in Chapter 4. The five well characterized MAGs—*Gracilimonas sediminicola* and *Microcella pacifica* recovered from *N. oceanica* IMET1 cultures, and *Algoriphagus aquaeductus*, *Erythrobacter* sp., and *Allorhizobium* sp. recovered from *T. obliquus* HTB1 cultures—encode for various genes involved in nutrient cycling, such as partial denitrification pathways as well as iron reduction. The two novel Patescibacteria—*Phycocordibacter aenigmaticus* and *Minuscolum obligatum*—that were recovered from cultures of both microalgae, are highly unusual in that they lack complete glycolysis and TCA pathways and lack machinery to synthesize any amino acids. Instead, they encode for the reductive pentose phosphate pathway (Calvin cycle) for essential biosynthesis and redox balance. *P. aenigmaticus* may also rely on elemental sulfur oxidation (*sdo*), partial nitrite reduction (*nirK*), and sulfur-related amino acid metabolism (SAME → SAH). The absence of full metabolic pathways for energy production in *P. aenigmaticus* and *M. obligatum* suggests extreme metabolic streamlining and obligate symbiosis with their algal hosts. This result was supported by data using the program, symcla, that predicts lifestyle and symbiosis traits for each MAG. Finally, genes that encode plant growth-promotion traits (PGPTs) were analyzed. Genes for plant-derived substrate usage were found to be encoded in the highest relative abundance across all samples, indicating bacteria may benefit from algal-secreted compounds. Further, plant vitamin production genes were found to be positively correlated with several bacteria including *Microcella pacifica*, *Gracilimonas* sp., and

Patescibacteria group bacteria, suggesting these bacteria may promote algal growth through a secretion of vitamins.

5.2 Introduction

5.2.1 Synergistic exchange between microalgae and bacteria

The benefits of harnessing microalgal-bacterial consortia for bioremediation technologies are just beginning to be discovered. While the use of microalgae for various efforts, such as carbon capture and wastewater treatment, has always unknowingly benefitted from the input from naturally occurring bacteria, research efforts are now specifically targeting the microalgal microbiome in hopes of enhancing positive outcomes. Particular attention has been paid to bacteria that can promote microalgal growth (Kim *et al.*, 2014; Toyama *et al.*, 2018; Palacios *et al.*, 2022). Long before the focus on microalgae and bacteria, research focused on microbial interactions in the plant rhizosphere to identify plant growth-promoting bacteria (PGPB)⁵ (Glick, 2012) and plant growth-promoting rhizobacteria (PGPR) (Bashan and Holguin, 1998, and references therein). Many PGPBs have been selected for studies with microalgae as similar growth promoting mechanisms can occur both within the rhizosphere and phycosphere (Palacios *et al.*, 2022)

A typical case of symbiosis between microalgae and bacteria is through mutualistic chemical exchange; the microalgae provide organic carbon compounds, and the bacterium (or bacteria) provides vitamins. For example, the vitamin B₁₂-dependent auxotroph, *Lobomonas rostrata* and the bacterium *Rhizobium loti* were able to survive when co-cultured in a medium with neither an organic carbon source nor vitamin B₁₂ (Kazamia *et al.*, 2012). This phenomenon has been demonstrated within several microalgal-bacterial cultures (Croft *et al.*, 2005; Grant *et*

⁵ Other terms in the literature include Plant-beneficial bacteria (PBB) (Bashan and Holguin, 1998) and Growth-Promoting Bacteria (Palacios *et al.*, 2022).

al., 2014). Because of their well-studied roles in the rhizosphere (Bloemberg and Lugtenberg, 2001), *Rhizobium* spp. has been shown to also be prominent members of the phycosphere. Kim *et al.* (2014) found that *Rhizobium* spp. were the most dominant symbiont of *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Scenedesmus* sp., and *Botryococcus braunii*. When each algal culture was reinoculated with cultivated *Rhizobium* sp., growth rates of algae increased ~11% on average and growth rates of *Rhizobium* sp. increased ~110% on average, confirming their beneficial symbiosis (Kim *et al.*, 2014).

Other functional taxa of interest have been in the Rhodobacteraceae family (particularly the Roseobacter clade) which can make up 2-15% of pelagic prokaryotic communities and are often shown to supply vitamin B₁₂ (Wienhausen *et al.*, 2022, and references therein). One bacterium, *Phaeobacter gallaeciensis*, has been shown to both enhance and inhibit the growth of *Emiliania huxleyi* depending on bloom age. *P. gallaeciensis*, a member of the Roseobacter clade, attached to the cell surface of *E. huxleyi* where they secreted IAA that promoted microalgal growth, but ultimately killed *E. huxleyi* (Segev *et al.*, 2016). Another study found that when *P. gallaeciensis* was grown in a pure culture with the addition of p-coumaric acid, a product released by aging *E. huxleyi*, the bacterium started to produce novel compounds (termed roseobacticides) that were able to lyse *E. huxleyi* cells (Seyedsayamdost *et al.*, 2011). The intermittent symbiosis between *P. gallaeciensis* and *E. huxleyi* serves as just one example of the complex relationships between microalgae and bacteria. Not only can interactions quickly change depending on culture age, but the symbiosis can also be highly species-specific; a bacterium can stimulate the growth of one microalga and inhibit the growth of another (Meyer and Pohnert, 2019; Jacob *et al.*, 2024).

5.2.2 Harnessing the power of microalgal-bacterial consortia

The growth promoting and antagonistic effects of bacteria can be harnessed in various bioremediation strategies. For example, algicidal bacteria are often used to manage harmful algal blooms (Coyne *et al.*, 2022). The synergistic benefits of microalgal-bacterial consortia have been studied within various biotechnologies including wastewater treatment, heavy metal and pollutant bioremediation, and biological carbon capture. For example, a study partly out of the Hill laboratory showed that a bacterial strain (designated HW001) was able to efficiently aggregate *N. oceanica* for easy harvesting (Wang *et al.*, 2016). Within wastewater treatment, Li *et al.* (2021) found that a coculture using *Scenedesmus (Tetradesmus) obliquus* and *Bacillus megaterium* was more efficient in treating biogas slurry compared with microalgae alone. Further, using microalgal-bacterial consortia in wastewater treatment has been studied for effective removal of nutrients municipal, agricultural and industrial wastewaters (reviewed by Li *et al.*, 2019). Several combinations of microalgae and bacteria have been proven to efficiently treat wastewaters of all origins, including aquaculture wastewater, swine wastewater, and dairy effluent (Q. Yu *et al.*, 2023; Malla *et al.*, 2024). Further, wastewater remediation by microalgae and bacteria has been found to be enhanced with the addition of CO₂, which is often generated by the wastewater treatment process itself (Ji and Liu, 2022). For instance, the microalga *Spongiochloris* sp. and its native microbiome effectively captures CO₂ gas that was produced during the petroleum wastewater treatment at a rate of 2.9205 g L⁻¹ day⁻¹ for 120 days (Abid *et al.*, 2017). When supplemented with an inoculum of unsterilized sewage and bubbled with 10% CO₂, *T. obliquus* quickly depleted nutrients (nitrogen and phosphorus) in the sewage while also growing much better compared with cultures bubbled with air (0.39 g L⁻¹ day⁻¹ vs. 0.14 g L⁻¹ day⁻¹, respectively) (Ma *et al.*, 2020). Some research has shown that attached microalgal-

bacterial consortia (biofilms) perform better than suspended communities (*i.e.* in a raceway pond or photobioreactor). Studies have found that that microalgal-bacterial biofilms achieved higher biomass (Ozkan *et al.*, 2012; Blanken *et al.*, 2014) and were more efficient at nutrient removal (Christenson and Sims, 2012). One major advantage of using microalgal-bacterial consortia is the value-added products that are gained during the bioremediation strategy, including microalgal biomass and lipids used in biofuel and nutraceuticals. Any slight enhancement of microalgal growth by bacteria would increase both the success of the bioremediation strategy as well as the yield of valuable secondary products. Insights into the functional genes found within the bacterial communities associated with microalgae are key to obtaining a holistic view of the microbial mechanisms behind various bioremediation strategies.

5.2.3 Necessity for metagenomic sequencing and functional gene analysis to optimize various biotechnologies

Traditional biotechnological applications of microalgae are often based on axenic monocultures and rely on strain-specific genome analysis. The growing interest in technologies that exploit the advantages of microalgal-bacterial consortia has now shifted the sequencing focus to metagenomic studies in order to uncover specific interactions between microalgae and bacteria. Characterizing the functional capacity of bacterial communities is essential for both understanding the ecology of microalgal cultures and for optimizing their use in biotechnology. This is particularly important to reveal hidden microbial diversity represented by species unable to be grown in the laboratory, which is essential for capturing the true diversity of bacterial communities within microalgal cultures (Zaytsev *et al.*, 2024).

When designing and optimizing microbial systems for bioremediation, the primary aim is often to utilize an already existing metabolic pathway, for example, utilizing the natural photosynthetic pathways of microalgae to capture carbon from smokestack flue gas (Lee *et al.*, 2024). Recently, the reverse ecology approach has been developed to infer the metabolic networks of all of the species in a microbial community through metagenomic data and thus predict function inputs into a mixed microbial system (Cao *et al.*, 2016). Metabolic gene annotation also helps to predict microbial lifestyles, energy capabilities, and potential symbiotic relationships without direct experimental validation. Zaytsev *et al.* (2024) reviewed the most promising directions for metagenomic studies specifically for microalgae and bacterial communities. The authors discussed that while studies using Nanopore long read sequencing of microalgal metagenomes have started to emerge only recently, elucidating both taxonomical composition and functional potential of bacterial communities will help optimize designs for microalga-bacterial based technologies for wastewater treatment and carbon capture (Zaytsev *et al.*, 2024). In addition to metagenomics, several multiomics studies have been conducted on microalgae-bacterial consortia, particularly for the bioremediation of wastewater sources (review by Kumar and Shukla, 2024). Apart from one study that conducted a metabolomic analysis on *T. obliquus* cultures supplemented with ciprofloxacin (Wang *et al.*, 2023a), no previous omics-based analyses have been conducted on the microalgal strains used in this dissertation.

5.2.4 Overview of this chapter

In this chapter, I analyze the same rounds of metagenomic sequencing that were analyzed in Chapter 4. The sequence data were analyzed in three ways. First, functional gene analysis was conducted on each of the seven metagenomic assembled genomes (MAGs) using both KEGG

and HMM-custom databases through the bioinformatic program, METABOLIC (Jonas *et al.*, 2025b [in review]). This analysis provides insights into the metabolism (*i.e.* ATP synthesis and amino acid synthesis) of each MAG as well as the genes they encode for nutrient cycles (*i.e.* nitrogen, iron, sulfur). GapMind was also used for annotation of amino acid biosynthesis genes to supplement the data from METABOLIC. Second, functional gene analysis was conducted on all metagenomic reads which included DNA from organisms for which a MAG was not recovered (Jonas *et al.*, 2025b [in review]). For pooled metagenomic reads, the PLant-associated Bacteria web resource (PLaBAs) was used to assign a functional annotation of plant growth-promotion traits (PGPTs). Finally, the symcla program was used to predict the lifestyle of each MAG and whether they may be classified as free-living, a host-associated symbiont, or an intracellular symbiont.

5.3 Methods

5.3.1 Metagenomics library preparation and sequencing

All methods for library preparation and sequencing are discussed in section 4.3.1 in Chapter 4. The samples and metagenomic sequencing rounds in this chapter are identical to those described in Chapter 4.

5.3.2 Metabolic pathway and lifestyle prediction

Prodigal (v2.6.3) in Normal mode was used to perform protein coding gene prediction, creating input .faa files (Hyatt *et al.*, 2010). The symbiont classifier, symcla (v0.1.0) was used to predict the lifestyle of each MAG, and bacteria are classified as Free-living, Symbiont;Host-associated, or Symbiont;Intracellular (<https://github.com/NeLLi-team/symcla>). To analyze the metabolic potential of each MAG, METABOLIC-G (v4.0) (Zhou *et al.*, 2022) was used by applying Hidden Markov models (HMMs) to various databases (KEGG, Uniref, Kofam Pfam and METBAOLIC Custom) and using an -m-cutoff of 0.75 resulting in KEGG modules being labeled as ‘present’ if 75% or more of the KEGG module steps are present. Heatmaps were created in R with pheatmap.

5.3.3 Annotation of amino acid biosynthesis pathways

In addition to the amino acid biosynthesis pathways annotated by METABOLIC-G, GapMind was used to describe the biosynthesis of 17 amino acids and of chorismate, which is a precursor of the aromatic amino acids (Price *et al.*, 2020). GapMind does not include the biosynthesis of the three amino acids: alanine, aspartate, and glutamate. After using Prodigal to perform protein coding gene prediction, translated genomes were uploaded to the GapMind web

server (<https://papers.genomics.lbl.gov/cgi-bin/gapView.cgi?set=aa>), queried against their custom database (updated in July 2025), and labeled with UniProt proteome identifiers.

5.3.4 Plant growth promotion gene analysis

Plant growth promotion gene analysis was conducted using the Morpheus platform and was outsourced to the company, Computomics (Tübingen Germany). For long-read data, quality control analysis was conducted before and after filtering NanoComp (v1.24.0) (De Coster and Rademakers, 2023). Quality output included summary statistics, comparison of average read length, and comparison of average base quality score. Long reads were filtered with the chopper v0.8.0 tool of NanoPack2 by applying a minimum Phred average quality score of 10 and a minimum read length of 500 bp. All filtered long reads were mapped against the NCBI nr protein database using Diamond (v2.1.8.162) and MEGAN6 (6UE_6_25_9) using parameters described previously (Bağcı *et al.*, 2021). Error-corrected reads and their annotation as GFF3 files were exported from MEGAN6. Bacterial functional counts are normalized to 332,434,816 aligned bases per sample using the MEGAN6 tool compute_comparison with a contamination filter for eukaryotic and viral content. Bar plots displaying function abundances show the top 30 most abundant taxa/functions at a chosen rank or level, per sample or group. If fewer than 30 taxa/functions were present, all are displayed. For Taxon2Function heatmaps, the top 50 taxa of a taxonomic rank were correlated with the top 50 functions of a functional level using a Spearman correlation.

5.4 Results

5.4.1 Metabolic prediction using protein-coding gene annotation with KEGG and HMM databases: *M. obligatum* and *P. aenigmaticus*

Metabolic capacity of all seven MAGs was predicted with METABOLIC-G which queries annotated proteins of each MAG against HMM databases (KEGG KOfam, Pfam, TIGRfam, and custom HMMs), resulting in KEGG modules (Figure 5.1) and a custom HMM hit profile (Figure 5.2) (Kanehisa and Goto, 2000; Zhou *et al.*, 2022). Two of these MAGs correspond to bacteria *M. obligatum* and *P. aenigmaticus* that were found associated with both *N. oceanica* IMET1 and *T. obliquus* HTB1 cultures. Figure 5.1 shows the absence of complete carbohydrate metabolism pathways in both *M. obligatum* and *P. aenigmaticus*, implying neither bacterium can utilize or produce sugars through central carbon metabolism. Both cannot fully oxidize organic carbon to CO₂ for energy generation through the TCA cycle and the lack of ATP synthesis genes indicates they rely on alternative electron donors for metabolic processes. Both bacteria, however, do contain genes for purine and pyrimidine biosynthesis. Neither bacterium was found to encode for any amino acids using METABOLIC-G queried against the KEGG databases (Figure 5.1). As some of the enzyme annotations in databases such as KEGG have been found to be incorrect (Schnoes *et al.*, 2009), Figures A5.1–A5.7 in the Appendix show an alternative method for predicting amino acid biosynthesis pathways using GapMind. Using GapMind against the Uniprot database, *M. obligatum* was found to encode for asparagine and glycine (Appendix, Figure A5.1) and *P. aenigmaticus* was found to encode for glycine (Appendix, Figure A5.2).

When analyzing individual genes that make up complete pathways for energy production (i.e. glycolysis), both *M. obligatum* and *P. aenigmaticus* encode for genes within the reductive pentose phosphate pathway (Calvin Cycle). *M. obligatum* encodes for 10 out of the 22 genes needed for a complete pathway, and *P. aenigmaticus* encodes for 12 out of 22 (Table 5.1). The incomplete pathway in both organisms suggests that the Calvin Cycle may not function for full carbon fixation but instead for the biosynthesis of reducing power, NADPH. Additionally, *P. aenigmaticus* encodes for *atpA* (V/A-type), one gene of four encoding for Complex V ATP synthase, indicating a potential alternative mode for ATP production (Figure 5.2). The *P. aenigmaticus* MAG is the only one that encodes for Complex V (ATP synthase: V/A-type H⁺/Na⁺-transporting ATPase) and encodes for no other complexes involved in oxidative phosphorylation. The *P. aenigmaticus* MAG possesses the gene *sdo* (sulfur dioxygenase) suggesting it can oxidize elemental S (S⁰) to sulfite (SO₃²⁻), but lacks upstream sulfur oxidation genes (i.e. *FccAB*, flavocytochrome c sulfide dehydrogenase) which would initially oxidize hydrogen sulfide (H₂S) (Figure 5.2). The *P. aenigmaticus* MAG also encodes for *nirK*, nitrite reductase (NO-forming) but lacks all other nitrogen reduction genes (i.e. *nirS*, *nosZ*) (Figure 5.2). Instead of full denitrification to N₂ gas, based on the MAG analysis, the bacterium only can reduce nitrite (NO₂⁻) to nitric oxide (NO). The *M. obligatum* MAG, on the other hand, encodes for no genes involved in S and N metabolism.

5.4.2 Metabolic prediction using protein-coding gene annotation with KEGG and HMM databases: *Gracilimonas sediminicola*, *Microcella pacifica*, *Algoriphagus aquaeductus*, *Allorhizobium* sp., and *Erythrobacter* sp.

Metabolic prediction was done on five well characterized MAGs—*Gracilimonas sediminicola* and *Microcella pacifica* recovered from *N. oceanica* IMET1 cultures, and *Algoriphagus aquaeductus*, *Erythrobacter* sp., and *Allorhizobium* sp. recovered from *T. obliquus* HTB1 cultures. These five MAGs, in contrast to the two MAGs for *M. obligatum* and *P. aenigmaticus*, encode for the complete pathways for the majority central carbohydrate metabolism processes. However, certain microbes seem to lack genes for certain modules based on the analysis of these five MAGs. For example, all five MAGs do not have a complete pathway encoded for the second carbon oxidation (oxaloacetate => 2-oxoglutarate) of the citrate/TCA cycle (Figure 5.1). Further, *Allorhizobium* sp. and *Microcella pacifica* lack genes in all steps in the TCA cycle. *Allorhizobium* sp. encodes for 6 out of 16 genes in the TCA cycle and *M. pacifica* encodes for 10 out of 16 (Table 5.1). *Gracilimonas sediminicola*, *M. pacifica*, and *Erythrobacter* sp. lack the complete set of genes for steps within the Entner Doudoroff pathway and the oxidative phase of the pentose phosphate pathway, with *Erythrobacter* sp. also lacking genes for pyruvate oxidation, the step that connects glycolysis and the TCA cycle (Figure 5.1). Similar patterns of lack of genes can be found within ATP synthesis pathways and amino acid biosynthesis genes; for example, all MAGs but *Erythrobacter* sp. lack complete pathways for NADH:quinone oxidoreductase and all but *Allorhizobium* sp. lack genes for cytochrome o ubiquinol oxidase (Figure 5.1). Further, all MAGs but *Algoriphagus aquaeductus* are missing genes for proline metabolism and all MAGs do not encode full pathways for tyrosine biosynthesis. Apart from *P. aenigmaticus* and *M. obligatum*, the MAG with the least genes

encoding for amino acid biosynthesis is *G. sediminicola* which encodes for only two complete pathways of the 16 pathways displayed in Figure 5.1. The amino acid auxotrophy of *G. sediminicola* found by HMM (Figure 5.1) was corroborated by results using GapMind (Figure A5.6). The *M. pacifica* MAG was also missing several genes for amino acid synthesis (Figures 5.1 and A5.7) Other notable gene absence includes the *M. pacifica* MAG not fully encoding for either lipid metabolism pathway. Notable gene presence includes the MAG for *G. sediminicola* encoding beta lactam resistance and that for *Allorhizobium* sp. encoding for cobalamin biosynthesis as well as genes involved in the urea cycle, namely the urease enzyme (Figures 5.1 and 5.2).

The heatmap in Figure 5.2 displays counts of genes involved in various biogeochemical processes. None of the five MAGs, with the exception of that from *Erythrobacter* sp., encode for any genes involved in carbon fixation. The *Erythrobacter* sp. MAG encodes for two genes involved in the 3 hydroxypropionate cycle (Figure 5.2). When looking specifically at nitrogen cycling genes, none of the MAGs encode for genes involved in anaerobic ammonia oxidation (anammox), N₂ fixation, or nitrite oxidation (Figure 5.2). Similar to *P. aenigmaticus*, *G. sediminicola* and *Allorhizobium* sp. MAGs encode for nitrite reductase (*nirK*). The *Allorhizobium* sp. and *A. aquaeductus* MAGs also encode for several genes involved in nitrite reduction: *nosZ*, *nirfH*, *nirfA* (*A. aquaeductus*), *nirK* and *norC* (*Allorhizobium* sp.), and *nirB*, *nirD*, and *norB* (both organisms). The *Allorhizobium* sp. MAG also encodes for two genes involved in nitrate reduction: *napA* and *napB* (Figure 5.2). All five MAGs encode for various lipid complexes involved in oxidative phosphorylation, with most MAGs encoding for two or more. Further, all five MAGs encode for various Complex IV (cytochromes), with *Allorhizobium*

sp. at the high end of the spectrum encoding several genes for four cytochromes and *A. aquaeductus* at the low end encoding only for cytochrome c oxidase, cbb3-type (Figure 5.2).

In sulfur cycling, *Allorhizobium* sp., *A. aquaeductus*, and *Erythrobacter* sp. (all symbionts of *T. obliquus*) encode for sulfur dioxygenase (*sdo*) with *Allorhizobium* sp. also encoding for genes involved in thiosulfate oxidation: S-sulfosulfanyl-L-cysteine sulfohydrolase (*soxB*), sulfur-oxidizing protein (*soxY*), and sulfane dehydrogenase subunit (*soxC*). All five MAGs encode for various genes within sulfur metabolism pathways and sulfur-related amino acid metabolism (Figure 5.2). For example, all five encode various copies for dimethyl-sulfide monooxygenase subunit B in DMSO metabolism and for genes involving the metabolism of other organic sulfur sources, *i.e.* *metH* (Homocysteine --> Methionine, Figure 5.2). All five MAGs also encode for several copies of genes involved in iron reduction (Figure 5.2).



Figure 5.1 Heatmap [presence (green)| absence (black)] displaying metabolic capacity of all seven MAGs predicted with METABOLIC-G (Zhou *et al.*, 2022) querying annotated proteins with the KEGG database. A complete pathway was labeled as present if 75% or more of the steps involved in that pathway were found to be present. Figure displayed with permission from Jonas *et al.* (2025b).

Table 5.1 Gene counts from major energy-producing pathways annotated with KEGG. Total amount of genes in the pathway are displayed under the pathway name with individual genes found within each MAG listed below. If pathway was 75% complete, it appears as ‘present’ in Figure 5.1. PPP= pentose phosphate pathway.

	Glycolysis Total genes (14)	Gluconeogenesis Total genes (7)	PPP Total genes (13)	TCA cycle Total genes (16)	Reductive PPP (Calvin cycle) Total genes (22)
	Genes present				
<i>Allorhizobium</i> sp.	14	6	13	6	14
<i>A. aqueductus</i>	14	7	13	12	16
<i>Erythrobacter</i> sp.	11	5	11	12	14
<i>G. sediminicola</i>	14	7	9	14	12
<i>M. pacifica</i>	13	7	12	10	16
<i>M. obligatum</i>	3	2	2	0	10
<i>P. aenigmaticus</i>	4	2	4	0	12

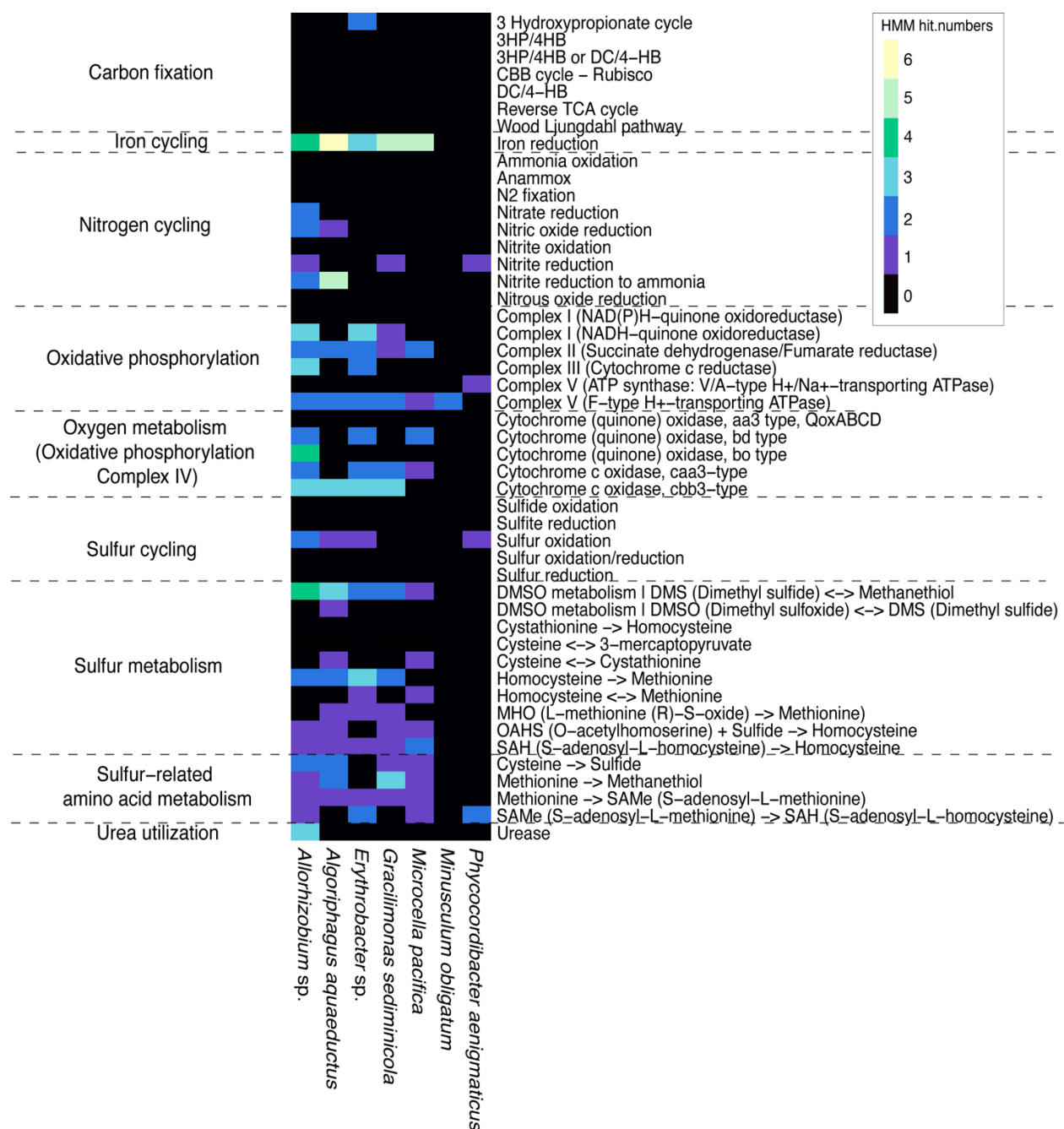


Figure 5.2 Heatmap displaying metabolic capacity of all seven MAGs predicted with METABOLIC-G (Zhou *et al.*, 2022) querying annotated proteins with Hidden Markov models (HMMs) resulting in custom HMM hit profiles. Individual gene counts within each pathway were summed. Figure displayed with permission from Jonas *et al.* (2025b).

5.4.3 Lifestyle predictions using symcla

The symbiont classifier, symcla, uses a machine learning-based approach to predict lifestyle based on 56 universal bacterial marker genes found in each MAG, UNI56. Scores assigned by symcla are interpreted as follows:

$\text{symcla_score} \leq 0.4$	= free-living
$0.42 < \text{symcla_score} < 1.21$	= symbiont (host-associated)
$\text{symcla_score} \geq 1.21$	= symbiont (intracellular)

M. obligatum and *P. aenigmaticus* both scored as being host-associated symbionts (0.487 and 0.548, respectively) and the remaining five classified MAGs scored as being free-living (all ≤ 0.003). (Figure 5.3, Table 5.2). Two bacteria, *Allorhizobium* sp. and *Erythrobacter* sp., scored negative, this being a common occurrence denoting free-living status.

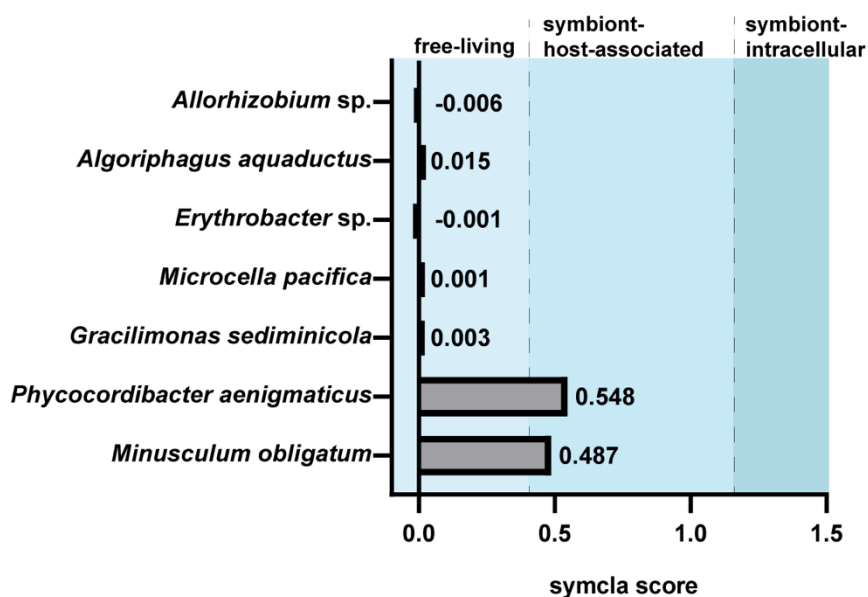


Figure 5.3. Symbiont scores of each MAG as determined by symcla. Light blue shading denotes a score correlated with free-living bacteria, medium blue denotes host-associated symbionts, and

dark blue denotes intracellular symbionts. Figure displayed with permission from Jonas *et al.* (2025b).

Table 5.2. Raw data output of the symcla lifestyle prediction model. UNI56: percentage of 56 universal bacterial marker genes, results >50% are considered valid. Feature gt01: number of features with a bitscore ≥ 0 . Feature ge20: number of features with a bitscore ≥ 20 . Feature ge100: number of features with a bitscore ≥ 100 . Confidence in symbiont prediction increases with UNI56 completeness and features gt01, ge20, and ge100.

MAG	Completeness UNI56	Features gt01	Features ge20	Features ge100	Symcla score	Microalgal host
<i>Minusculum obligatum</i>	91.07	175	107	33	0.487	<i>N. oceanica</i>
<i>Phycocordibacter aenigmaticus</i>	91.07	189	121	40	0.548	<i>N. oceanica</i>
<i>Gracilimonas</i> sp.	100	497	335	141	0.003	<i>N. oceanica</i>
<i>Microcella pacifica</i>	96.43	353	242	99	0.001	<i>N. oceanica</i>
<i>Erythrobacter</i> sp.	98.21	420	321	134	-0.001	<i>T. obliquus</i>
<i>Algoriphagus aquaeductus</i>	100	496	344	154	0.015	<i>T. obliquus</i>
<i>Allorhizobium</i> sp.	87.50	511	388	182	-0.006	<i>T. obliquus</i>

5.4.4 Functional gene analysis using PGPT databases

In previous sections, 5.4.1 and 5.4.2, metabolism predictions were constructed by examining each MAG individually. For this section, reads were pooled from various metagenomic sequencing rounds: MAG1_Round1 (*N. oceanica*), MAG1_Round2 (*N. oceanica*),

MAG3 (*N. oceanica*), and MAG5 (*T. obliquus*) (Table 4.2 | Chapter 4). This method was chosen to understand functional contributions of the system as opposed to individual bacteria. Reads were translated *in silico* on all open reading frames and annotated with the PLant-associated Bacteria web resource (PLaBAs), which assigns a functional annotation of plant growth-promotion traits (PGPTs) (Patz *et al.*, 2021).

Figure 5.4 shows that relatively abundant PGPT Level 3 functions within three cultures of *N. oceanica* and one culture of *T. obliquus*. While PGPT Levels 1 and 2 display general categories of function genes, *i.e.* “Direct Effects” (Level 1) or “Bioremediation” (Level 2), Level 3 starts to elucidate more detailed bacterial functionality. The PGPT Level 3 functional genes found within the bacterial communities of both microalgae were relatively consistent with the most abundant functions being Competitive Exclusion (CE) Bacterial Fitness, CE Bacteria Secretion, CE Cell Envelope Remodeling, CE Quorum Sensing Response, Colonization-Plant Cell Wall/Membrane Degradation, Colonization- Plant Derived Substrate Usage, Heavy Metal Detoxification, Neutralizing Abiotic Stress, Plant Vitamin Production, Phosphate and Potassium Solubilization, and Universal Stress Response (Figure 5.4). The PGPT Level 3 annotation with the highest relative abundance in all three sequencing rounds was Plant Derived Substrate Usage (Figure 5.4 – royal blue bars). A Spearman correlation was conducted between the top species and top functions (Taxon2Function) found within three pooled *N. oceanica* sequencing rounds (Figure 5.5). Similar PGPT Level3 Functions were found to be highly correlated (>0.5 correlation value) with various taxa found within *N. oceanica* cultures. For example, Plant Signal-Germination Stimulation, Neutralizing Abiotic Stress, Potassium and Phosphate Solubilization, Fluoride Detoxification, Colonization-Surface Attachment, Plant Vitamin Production, Competitive Exclusion-Bacterial Secretion, and Universal Stress Response genes

were positive correlated with the presence of *Balneola* sp., *Micrococcales* sp., *Microcella indica* (*pacifica*), *Microbacterium enclense*, *Cryobacterium* sp. Y62, *Clavibacter michiganensis*, *Parcubacteria* bacteria, *Gracilimonas* sp., *Candidatus* Kaiserbacteria bacteria, and *Patescibacteria* group bacteria (Figure 5.4). Further, Fluoride Detoxification and Colonization-Surface Attachment genes had the highest correlation with *Microcella indica* (*pacifica*), *Microbacterium enclense*, *Cryobacterium* sp. Y62, and *Clavibacter michiganensis*. Finally, Plant Vitamin Production genes had the highest correlation with *Parcubacteria* bacteria, *Gracilimonas* sp., *Candidatus* Kaiserbacteria bacteria, and *Patescibacteria* group bacteria (Figure 5.4). A Taxon2Function analysis was unable to be conducted on *T. obliquus* cultures as there needed to be three or more samples in the analysis; the correlation heatmaps would only display correlation values of +1 (present) and -1 (absent). Only one successful metagenomics sequencing run was recovered from *T. obliquus* cultures (Table 4.2 | Chapter 4).

PGPT Level 4 functions were not chosen for further analysis due to redundancies with PGPT Level 5, the latter giving more useful information as it pertained to our microalgal system. There were several functions annotated as PGPT Level 5 functions across both *N. oceanica* and *T. obliquus* samples with a high level of evenness (Figure 5.6). Level 5 PGPT functions included those relating to bacterial fitness (Transposases), Plant-derived Purine Metabolism, and Plant Vitamin Production (Folate and Pantothenate) (Figure 5.6). Other functions present include Heat Shock Proteins, Arylopolyyene Biosynthesis, Halotolerance-related enzymes and genes related to DNA Damage Defense and Repair (Figure 5.6)

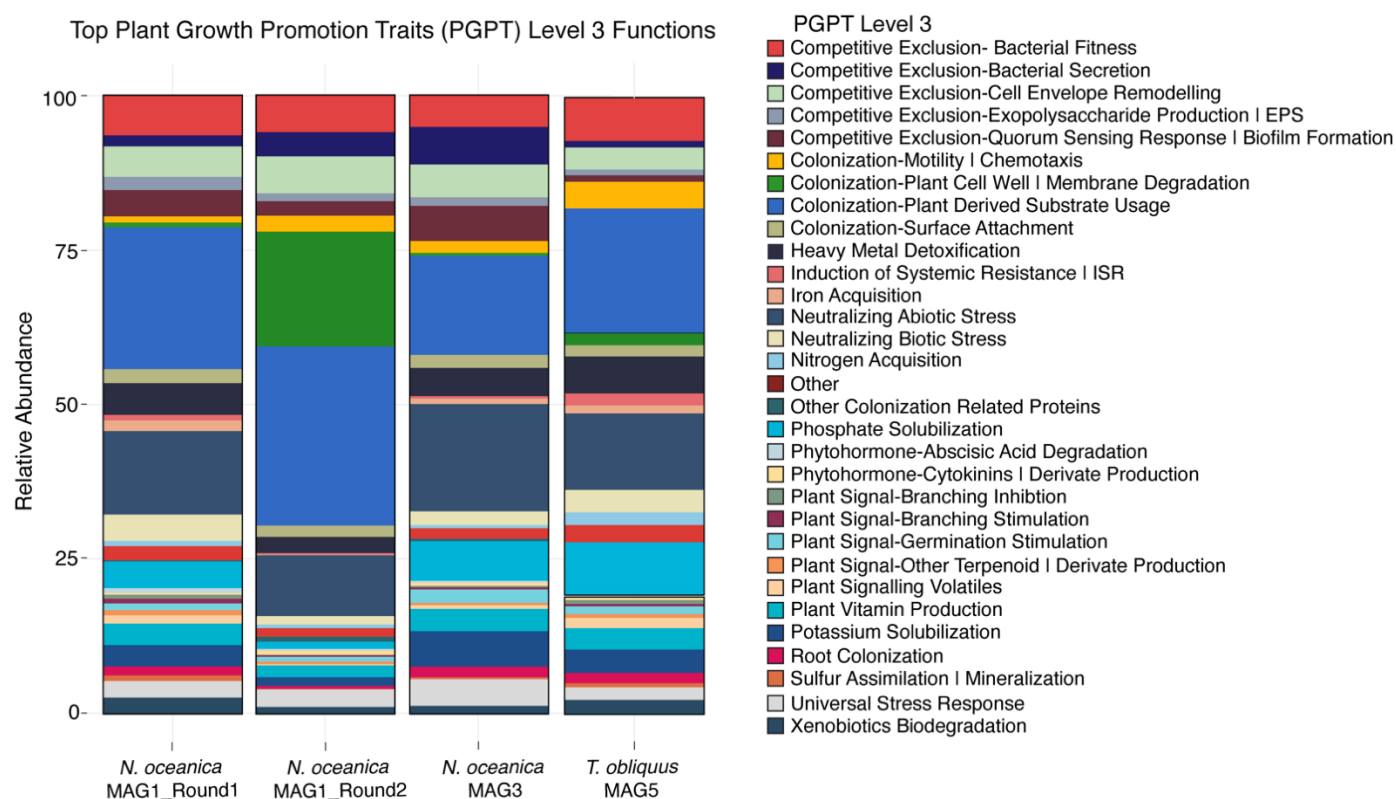


Figure 5.4 Functional gene analysis from three rounds of metagenomic sequencing of *N. oceanica* cultures and one round of sequencing of *T. obliquus* cultures. Stacked bar charts displaying the most abundant functions within plant growth-promotion traits (PGPT) Level 3. Details of metagenomic sequencing rounds are displayed in Table 4.2. in Chapter 4.

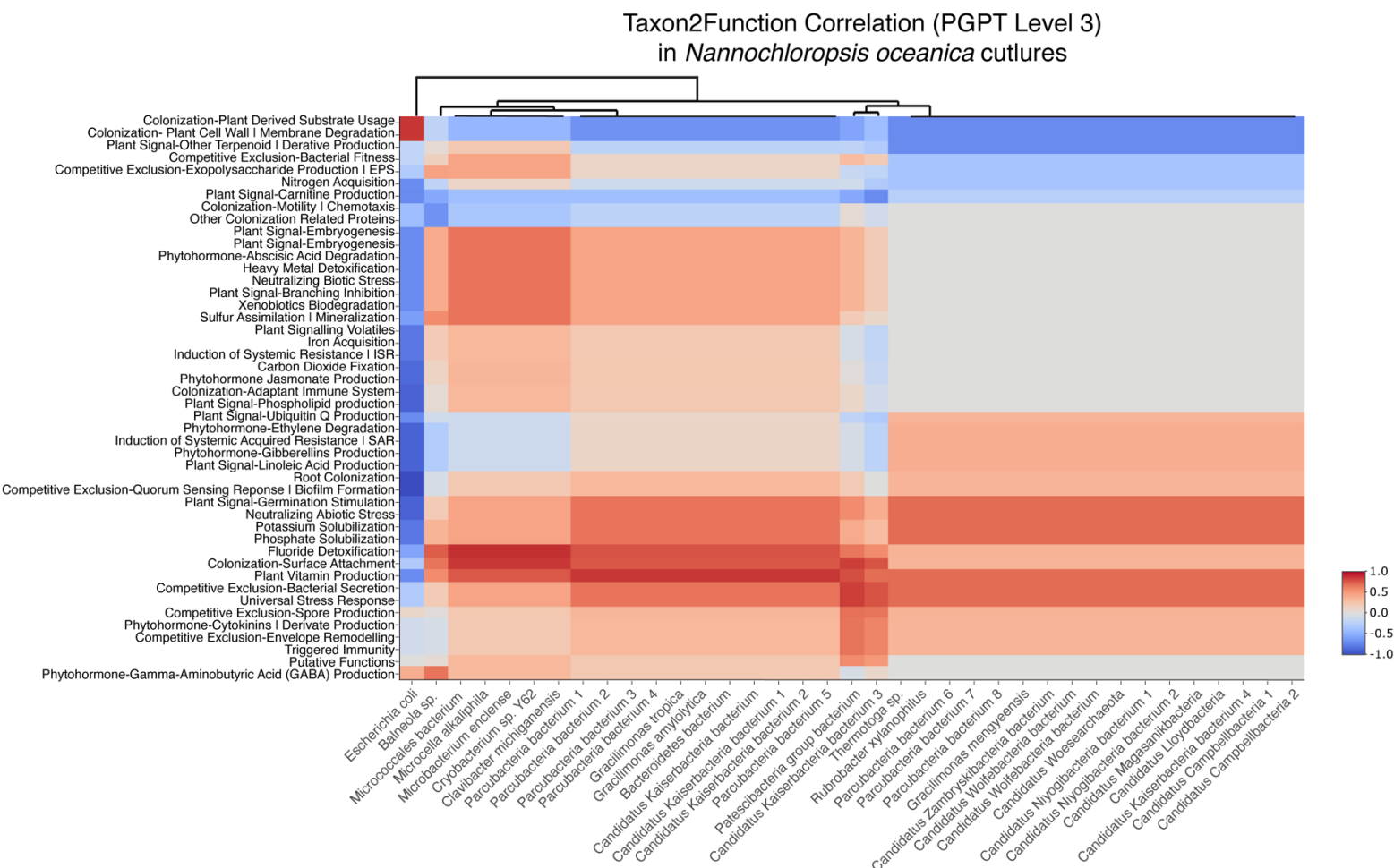


Figure 5.5 Heatmap displaying the correlation values of the most abundant species with the top functions within PGPT Level 3 using Spearman's rank correlation coefficient. Three sequencing rounds of *N. oceanica* (MAG1_Round1, MAG1_Round2, and MAG3) were pooled. Some strain names have been abbreviated (e.g. Paracubacteria bacterium1) and a table displaying the current full name of organisms in NCBI is shown in Table 5.3 on the following page. Figure displayed with permission from Jonas *et al.* (2025a).

Table 5.3 Table displaying the NCBI's current full name of organisms abbreviated in heatmap in Figure 5.5.

Label in Figure	Organism full name
Parcubacteria 1	Parcubacteria group bacterium GWA1-56-13
Parcubacteria 2	Parcubacteria group bacterium GW2011-GWA1-47-8
Parcubacteria 3	Parcubacteria group bacterium Athens-0714-26
Parcubacteria 4	Parcubacteria bacterium C7867-004
Parcubacteria 5	Parcubacteria group bacterium GW2011-GWA2-42-18
Parcubacteria 6	Parcubacteria group bacterium GW2011-GWA1-54-9
Parcubacteria 7	Parcubacteria group bacterium 21-54-25
Parcubacteria 8	Parcubacteria bacterium C7867-001
Candidatus Kaiserbacteria 1	Candidatus Kaiserbacteria bacterium 21_14_0_10_47_16
Candidatus Kaiserbacteria 2	Candidatus Kaiserbacteria bacterium 21_14_0_10_49_17
Candidatus Kaiserbacteria 3	Candidatus Kaiserbacteria bacterium OXYB1_FULL_46-14
Candidatus Kaiserbacteria 4	Candidatus Kaiserbacteria bacterium 21_14_0_10_45_20
Candidatus Niyogibacteria 1	Candidatus Niyogibacteria bacterium O2_12_FULL_41_13
Candidatus Niyogibacteria 2	Candidatus Niyogibacteria bacterium 21_14_0_10_46_36
Candidatus Campbellbacteria 1	Candidatus Campbellbacteria 21_14_0_10_35
Candidatus Campbellbacteria 2	Candidatus Campbellbacteria OXYC2_FULL_35_25

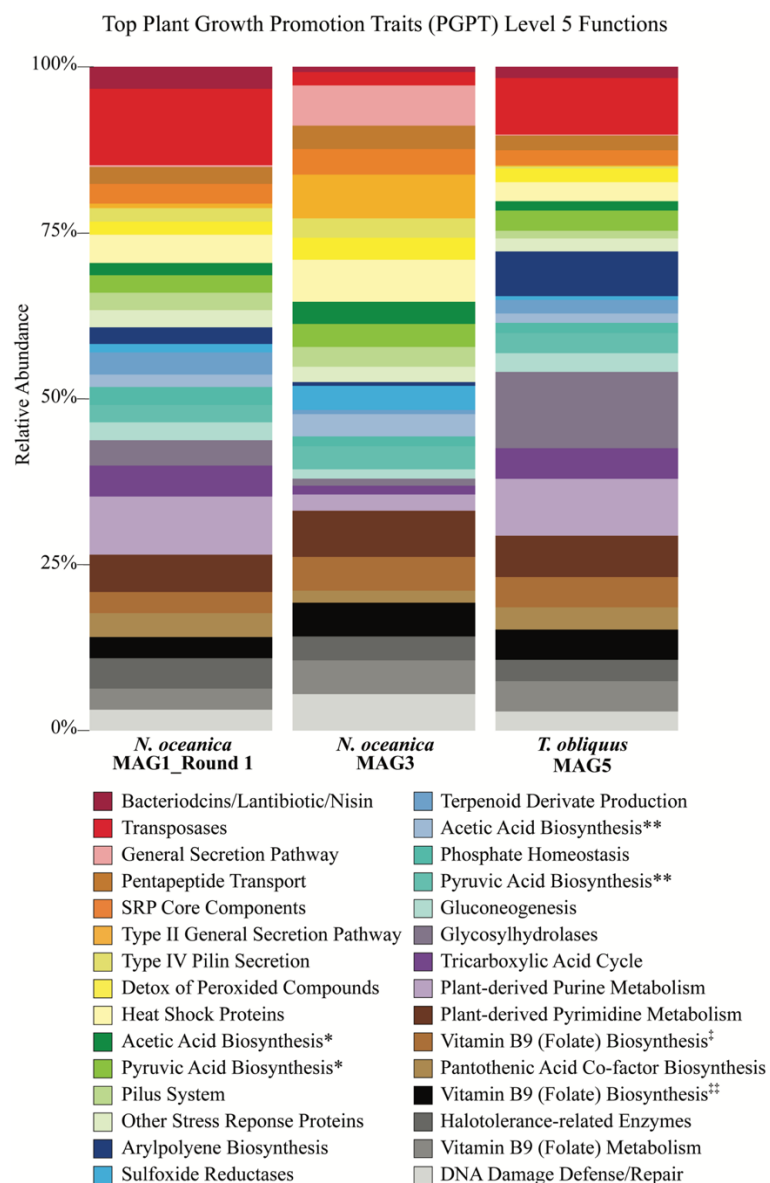


Figure 5.6 Functional gene analysis from bacteria associated with *N. oceanica* IMET1 and *T. obliquus* HTB1. Stacked bar charts displaying the most abundant functions within plant growth-promotion traits (PGPT) Level 5, functions classified as “Other” have been removed. *K solubilization Acetic acid. **P solubilization Acetic acid. ‡ Plant Germination Folate Biosynthesis. ‡‡ Plant Vitamin Folate Biosynthesis. Details of metagenomic sequencing rounds are displayed in Table 4.2 in Chapter 4.

5.5 Discussion

5.5.1 Nitrogen, sulfur, and iron metabolism within the *T. obliquus* and *N. oceanica* phycosphere

The five previously characterized MAGs recovered in this dissertation—*Gracilimonas sediminicola*, *Microcella pacifica*, *Algoriphagus aquaeductus*, *Erythrobacter* sp., and *Allorhizobium* sp.—encode for metabolic capabilities across various nutrient cycles. For example, *Allorhizobium* sp. belongs to a larger clade (*Allorhizobium*–*Neorhizobium*–*Pararhizobium*–*Rhizobium*) that are well-known members of microbial communities that mediate nitrogen cycling in soils and plant root systems (De Lajudie et al., 1998). *Allorhizobium* sp. and *A. aquaeductus* (both associated with *T. obliquus*), encode for many denitrification genes, although neither MAG encodes for the four enzymatic steps in the complete pathway. *Allorhizobium* sp. contains genes (*napA* and *napB*) for nitrate reduction (first step in denitrification), for three nitrite reduction genes *nirB*, *nirD*, and *nirK* (second step) as well as *norB* and *norC*, encoding for nitric oxide (NO) reductase that is involved in the third step of denitrification, NO reduction to nitrous oxide (N₂O) (Figure 5.2). However, it does not encode for nitrous oxide reductase (*nosZ*), which reduces N₂O to N₂ and is the fourth and final step of the denitrification pathway. A neighboring bacterium in *T. obliquus* cultures, *A. aquaeductus*, does encode for *nosZ*, completing the denitrification pathway. *A. aquaeductus* also encodes for the enzymes involved second and third steps (nitrite reductase, *nirB* and *nirD*, and nitric oxide reduction, *norB*), but does not encode for the first step in denitrification, nitrate reduction. However, nitrate reduction has been reported previously for several *Algoriphagus* species (J. R. Han et al., 2017; Kim et al., 2020; W. Liu et al., 2021).

While most denitrifiers encode either *nirK* or *nirS* (Salas *et al.*, 2021), it has shown that *Bacillus nitroreducens* and *Bradyrhizobium oligotrophicum* (in the same order as *Allorhizobium* sp.) contain genes for both nitrite reductases as *A. aqueductus* (Jang *et al.*, 2018; Sánchez and Minamisawa, 2018). As enzymes for a complete pathway are not encoded within one bacterium, there may exist shared redox reaction within the *T. obliquus* phycosphere. This shared interaction may include *P. aenigmaticus*, whose highly reduced genome has retained the gene *nirK* for nitrite reduction and would depend on neighboring organisms, such as *Allorhizobium* sp. and *A. aqueductus* (and potential others) to further reduce the toxic NO product. *Gracilimonas sediminicola*, a member of *N. oceanica* microbiome, also encodes for *nirK* and no other nitrogen cycling enzymes. Apart from *nirK* encoded by *G. sediminicola* and *P. aenigmaticus*, no other nitrogen cycling genes were found in the metagenomes recovered from *N. oceanica* cultures (Figure 5.2). Another absence from both microalgal cultures was genes involved in nitrogen fixation, which is surprising given that *Allorhizobium* species have been characterized within nitrogen-fixing consortia previously (De Lajudie *et al.*, 1998; Mousavi *et al.*, 2014).

Allorhizobium sp., *A. aqueductus*, *P. aenigmaticus* as well as the third bacterium associated with *T. obliquus*, *Erythrobacter* sp. all encode for sulfur dioxygenase (*sdo*), which oxidizes elemental S (S^0) to sulfite (SO_3^{2-}) (Figure 5.2). All four do not encode for any other S cycling genes, neither upstream sulfide oxidation nor downstream sulfur reduction. *Allorhizobium* sp. was the only MAG to encode for thiosulfate oxidation in this study; however *Erythrobacter flavus* found in a deep-sea cold seep was found to also encode for this pathway (Zhang *et al.*, 2020). The *Erythrobacter* sp. of this study encoded for *sdo* and no other sulfur cycling genes. All five of the well-characterized MAGs encode for several genes for sulfur metabolism, including dimethyl sulfoxide (DMSO) and other organic sulfur compounds (Figure

5.2). DMSO metabolism is often observed in facultative anaerobic or anaerobic bacteria and is as of yet understudied within the bacterial communities of microalgae (Wang *et al.*, 2021). Several studies, however, have focused on the levels of DMSO and dimethylsulfoniopropionate (DMSP) within microalgal cell contents (Speeckaert *et al.*, 2019, and references therein). None of the five MAGs have been involved in a study that focused on sulfur metabolism. A recent *Algoriphagus* sp. MAG did encode for genes involved in sulfur cycling (Kim *et al.*, 2020), although the genes cannot be directly compared to that of this study as the authors used a different database (Enzyme Commission Number). The genes with the highest copy number in this study were those encoding for iron reduction (Figure 5.2). Again, these genes were encoded by the five well-characterized MAGs and not by *M. obligatum* and *P. aenigmaticus*. Iron availability is well researched topic within microalgae and bacteria as the latter can produce siderophores that ensure iron availability for their hosts and can promote microalgal growth rates (Rawat *et al.*, 2022). Although gene presence is high for iron reduction in this study, there were no genes found that are involved in siderophore production. The most relevant absence from the MAGs of this dissertation are any genes related to carbon fixation. Besides two genes encoding for the 3-Hydroxypropionate cycle in *Erythrobacter* sp., no genes related to carbon fixation were found. However, these bacteria may still contribute positively to our carbon capture system indirectly through growth enhancement of *N. oceanica* or *T. obliquus*.

Allorhizobium sp. encodes for gene involving urease utilization (Figure 5.1), which could be of interest in the context of microbially induced carbonate precipitation (MICP). Under alkaline conditions, the urease enzyme catalyzes the hydrolysis of urea into ammonia (NH₃) and CO₃²⁻. The CO₃²⁻ can be precipitated in the presence of calcium to form CaCO₃. The precipitation of CaCO₃ has been observed in both algal systems of this study (Lee *et al.*, 2024;

Chen laboraotry unpublished data). There are two possibilities as to how *Allorhizobium* sp. contribute to MICP, the first being that the bacteria help microalgal growth as seen across multiple studies (Palacios *et al.*, 2022). The second possibility is that the bacteria themselves are contributing to CaCO₃ precipitation. The literature indicates *Gracilimonas* sp. and *Microcella* sp. are often found in carbonate formations (Skorupa *et al.*, 2019), indicating they could be key players within our system, but we cannot claim this is occurring in our system as of yet.

5.5.2 Amino acid auxotrophy prevalent in both *M. obligatum*, *P. aenigmaticus*, and the five well-characterized MAGs

The inability to synthesize certain amino acids, amino acid auxotrophy, has been found to be widespread across bacterial phyla. Ramoneda *et al.* (2023) estimates 78% of taxa are able to synthesize all amino acids and that auxotrophies are more common within obligate intracellular parasites and within free-living taxa with genomic characteristics suggesting streamlined lifestyles. Auxotrophy can be particularly advantageous within nutrient rich laboratory cultures where essential metabolites are available from the surrounding environment or from nearby organisms (Zengler and Zaramela, 2018). *G. sediminicola* and *M. pacifica* were found to have various missing genes for the synthesis of amino acids. The GapMind annotation displayed a high confidence for only three complete amino acids gene sets in *M. pacifica* (Appendix, Figure A5.7) and four in *G. sediminicola* (Appendix, Figure A5.6). The genome of *Gracilimonas tropica* (isolated from a *Synechococcus* culture) displayed auxotrophy for eight amino acids (lysine, phenylalanine, tyrosine, arginine, aspartic acid, isoleucine, proline, and methionine) (Choi *et al.*, 2015). However, when testing auxotrophy by growing *G. tropica* in minimal media with the absence of certain amino acids, the strain was only found to be auxotrophic for isoleucine and methionine (Choi *et al.*, 2015). This finding indicates that bacterial amino acid

auxotrophy cannot be confirmed until growth experiments have taken place and that the auxotrophy found within *G. sediminicola* and *M. pacifica* may be strictly a genomic characteristic. Additionally, genome completeness has been shown to contribute a significant bias on recovered functional signal, and metagenomes can often display incomplete metabolic pathways compared to isolate genomes due to low completeness (Zhou *et al.*, 2022; Eisenhofer *et al.*, 2023). Thus, I acknowledge that the functional capacities presented in Figures 1 and 2 may be underestimated. This could be particularly true for *M. pacifica* (completeness = 81.33%) and *A. aquaeductus* (completeness = 94.45%) (Table 4.4 | Chapter 4).

Along with the typical absence of complete pathways for central carbohydrate metabolism, both *M. obligatum* and *P. aenigmaticus* also lack all amino acid biosynthetic pathways when looking at KEGG annotation (Figure 5.1). This finding is striking and in agreement with recent studies with the exception of that of Haro-Moreno *et al.* (2023), who reported many amino acid biosynthetic routes found within a collection of Patescibacteria, especially within their recovered MAGs from Lake Baikal (Haro-Moreno *et al.*, 2023). When annotating proteins with GapMind, *M. obligatum* was found to encode for glycine and asparagine biosynthesis (Figure Appendix, A5.1), despite its genome encoding 35 tRNAs for all amino acids except for asparagine (Section 4.4.2 | Chapter 4). The complete amino acid auxotrophy within highly reduced genomes has been previously recorded (Suthers *et al.*, 2009), especially among Patescibacteria whose highly reduced genomes make them dependent on external sources of amino acids and energy. Not only do Patescibacterial MAGs often display complete amino acid auxotrophy, but also commonly lack genes for the biosynthesis of nucleotides, vitamins, and lipids (Nelson and Stegen, 2015).

5.5.3 Metabolic reconstruction reveals extreme streamlining within *M. obligatum* and *P. aenigmaticus*

The reduced genome sizes of *M. obligatum* (578,798 bp) and *P. aenigmaticus* (721,362 bp) lead to a hallmark feature shared among Patescibacteria, limited metabolic capabilities (Rinke *et al.*, 2013; Brown *et al.*, 2015; Castelle *et al.*, 2018). Neither *M. obligatum* nor *P. aenigmaticus* encode for complete pathways for glycolysis, gluconeogenesis, the pentose phosphate pathway, the TCA cycle, and ATP synthesis (Figure 5.1). Taking into consideration the absence of key metabolic genes for energy production along with their symcla scores between 0.42–1.21 (Figure 5.3), *M. obligatum* and *P. aenigmaticus* are most likely obligate symbionts of *T. obliquus* HTB1 and *N. oceanica* IMET1. One gene of interest encoded by *P. aenigmaticus*, *nirK*, provides some evidence for preserved metabolic function across Patescibacteria as it is found within various lineages and thought not to be acquired by horizontal gene transfer (Danczak *et al.*, 2017; He *et al.*, 2021). This function could be a strategy for managing nitrite toxicity, especially in the presence of a neighboring organism able to further reduce nitric oxide such as *Allorhizobium* sp. and *A. aquaeductus* (Figure 5.2). *P. aenigmaticus* possesses the gene *sdo* (sulfur dioxygenase) but lacks upstream sulfur oxidization genes. Instead, *P. aenigmaticus* likely relies on sulfur within the algal growth medium as a terminal electron acceptor with the oxidation reaction generating NADPH to support other biosynthetic processes. Three other Patescibacteria have been shown to encode *sdo* as well as other genes involved in sulfate reduction, *sat*, *cysC* and *cysN*, all three of which were not encoded by *M. obligatum* and *P. aenigmaticus* (He *et al.*, 2021). However, *cysN* and *cysD* were encoded by other four MAGs recovered from our system, implying there might exist shared redox reactions within bacterial communities as may be the case with nitrite and nitric oxide reduction.

Without complete pathways encoding for glycolysis, gluconeogenesis, or the TCA cycle, Patescibacteria generally cannot generate energy or biosynthetic intermediates from organic carbon. Moreover, absence of complete ATP synthesis pathways indicates that oxidative phosphorylation pathways are not utilized to generate energy in these bacteria. The only exception was one gene, *atpA* (V/A-type) of Complex V ATP synthase, encoded by *P. aenigmaticus* (Figure 5.2). Instead, *M. obligatum* and *P. aenigmaticus* encode for a part of the reductive pentose phosphate (Calvin Cycle) pathway, genes of which have been found in other Patescibacteria including in the Paceibacteria class (Table 5.1) (Chaudhari *et al.*, 2021; Chiriac *et al.*, 2022). However, the incomplete pathway suggests it is not functional for complete CO₂ fixation and metabolic independence. The presence of genes encoding for Calvin Cycle machinery, albeit incomplete, suggests their ability to produce NADPH and potentially ribose-5-phosphate nucleotides. Therefore, both are likely obligatory symbionts that depend on essential metabolites from algal hosts, other bacteria within the system, or from scavenging compounds within the algal growth medium and/or phycosphere. This observation aligns with adaptations observed in intracellular symbionts and bacteria with highly streamlined genomes (Driscoll *et al.*, 2017). I ascertain both bacteria are highly obligate symbionts, but not endosymbiotic within the microalgae based on their symcla scores (Figure 5.3) and ability to pass through a 0.45 µm filter (Figure 3.9 | Chapter 3). As metabolic reconstruction relies on the presence/absence of genes and not actual metabolites, it is only an inference into an organism's actual metabolism. To prove the metabolic capabilities and preferences of a bacterium, one must conduct a metabolomic analysis through gas chromatography and mass spectrometry. This type of analysis can only be conducted on organisms that are able to be cultivated in the laboratory. Pure cultures of Patescibacteria have yet to be achieved. Some have been cocultured but this has also proven

quite difficult, as the hosts must also be cultivated and maintained (He *et al.*, 2015; Yakimov *et al.*, 2022).

There is a likelihood that both *M. obligatum* and *P. aenigmaticus* are symbionts of other microalgae-associated bacteria rather than of the microalgae themselves, based on their prevalence in the free-living (0.22 μm) fraction rather in the microalga-associated (0.45 μm) fraction. Symbiosis between Patescibacteria and a bacterial host has been described previously (He *et al.*, 2015). Additionally, two CPR bacteria, a member of Saccharibacteria (formerly known as TM7) and *Vampirococcus*, are both obligate, parasitic epibionts of the bacteria *Actinomyces odontolyticus* and *Halochromatium* sp., respectively (He *et al.*, 2015; Moreira *et al.*, 2021). In previous work, we found *M. obligatum* associated with *N. oceanica* at the 1 L culture scale in the closely associated fraction (0.45 μm) in similar relative abundance to free-living fraction (0.22 μm) (Lee *et al.*, 2024; Jonas *et al.*, 2025a). We have never found either Patescibacterium solely in the microalgae cell-associated (0.45 μm) fraction, strongly suggesting that the Patescibacteria are symbionts of other bacteria rather than the microalgae.

5.5.4 Bacterial contribution to microalgal systems: Plant Growth Promotion Traits (PGPTs)

PGPT Level 3 (Figures 5.4 and 5.5) and Level 5 assignments (Figure 5.6) were insightful for understanding the role of bacteria on carbon capture through microalgal growth enhancement. There were no relevant findings when looking at bacterial genes related to carbon fixation, although we hypothesize bacterial symbionts may contribute to microalgal growth enhancement through mechanisms such as vitamin production and secretion (Figures 5.5 and 5.6). For example, genes for folate (vitamin B₉) and pantothenate (vitamin B₅) biosynthesis were

found in bacterial metagenomic sequence data from both microalgal cultures of this study (Figure 5.6). This corroborates findings of genes reported within the algal phycosphere (Zhu *et al.*, 2024) as well as bacterial symbionts of microalgae (Cho *et al.*, 2020). For example, Astafyeva *et al.* (2022) found that genes for riboflavin (B₂) and pyridoxine (vitamin B₆) were highly expressed in *Porphyrobacter* (synonymous genus of *Erythrobacter*), a symbiont of *Scenedesmus quadricauda*. Further, *Roseibium* sp. (RMAR6-6) encoded for complete gene sets for numerous B vitamins, including thiamine (B₁), riboflavin (B₂), pantothenic acid (B₅), pyridoxine (B₆), folic acid (B₉) and cobalamin (B₁₂) (Kim *et al.*, 2024). Interestingly, genes for cobalamin (B₁₂) biosynthesis were found in *Allorhizobium* sp. in this study. Several algal strains are known to be vitamin B auxotrophs, suggesting their need to obtain these vitamins from the environment or bacterial symbionts (Croft *et al.*, 2006). Therefore, riboflavin and pyridoxine produced by the bacteria of this study could be provided to their algal hosts in exchange for algal-derived substrates, such as purine and pyrimidine (Figure 5.6). This hypothesis is supported when zooming out to PGPT Level 3 annotation, where the function with the highest relative abundance in all three sequencing rounds was Plant Derived Substrate Usage (Figure 5.4).

Algae are known to release dissolved organic carbon (DOC) into the water that can then be consumed by bacteria within the phycosphere. Many microalgal cultures are maintained in co-culture with bacteria due to the potential for bacteria to promote algal growth. These bacteria have been termed microalga growth-promoting bacteria (MGPB) with examples having been provided in Chapter 1 (Palacios *et al.*, 2022). Although several PGPT traits were encoded for, no phytohormone-related genes were found in this study. The function, Fluoride Detoxification, was positive correlated with the presence of *Balneola* sp., *Micrococcales* sp., *Microcella indica* (*pacifica*), *Microbacterium enclense*, *Cryobacterium* sp. Y62, *Clavibacter michiganensis*,

Parcubacteria bacteria, *Gracilimonas* sp., *Candidatus* Kaiserbacteria bacteria, and Patescibacteria group bacteria (Figure 5.5). This is consistent with the fact that fluoridated tap water is used to prepare algae medium at Hytek Bio where sample MAG1_Round2 originated from.

The bacteria that seem to contribute the most functionality to *N. oceanica* cultures are *Balneola* sp. and the members of the Patescibacteria group. Any taxa labeled as ‘Patescibacteria group’ are considered to be *M. obligatum* and *P. aenigmaticus*. Further, any taxa labeled as “*Candidatus* Kaiserbacteria” and “Parcubacteria” could be the same organisms as “Patescibacteria group bacterium.” This is challenging to confirm due to the current phylogenetic difficulty of classifying members of Patescibacteria within public databases. As I am the first to classify *M. obligatum* and *P. aenigmaticus*, their names are still ambiguous in prior taxonomic and functional gene databases. Not only do *M. obligatum* and *P. aenigmaticus* contribute the most function to the system, but they are also the most abundant taxa found in the 16S rRNA gene sequencing data at both the 1 L and 500 L scale (Figures 3.9 and 3.10 | Chapter 3). Many studies have shown that Patescibacteria often attach to the surface of other bacteria, hence the high correlation with Colonization-Surface Attachment genes (Figure 5.5) (Kuroda *et al.*, 2022; Gios *et al.*, 2023). There also was a high correlation with Bacterial Secretion, Plant Vitamin Production, and Universal Stress Response. The dominance and functional contribution of Patescibacteria, namely *M. obligatum* and *P. aenigmaticus*, in our microalgal cultures warrants their further investigation as key symbionts that may impact algal growth and/or carbon sequestration efficiency. The most impactful way to find evidence of their functional contributions would be through transcriptomic, proteomic, and metabolomic analysis.

Chapter 6: Conclusions and Future Directions

6.1 The big picture: Improving the efficiency of biologically mediated carbon capture systems

The combustion of fossil fuels, among other human-centric activities, has led to an increase of greenhouse gases in our atmosphere, including CO₂. Increased CO₂ in the atmosphere has led to a wide range of environmental problems that have affected ecosystem functioning as well as weather and climate patterns. As such, “sustainability” and “carbon capture” have become important concepts in our society as we search for solutions to decrease atmospheric CO₂ and try to reduce the impacts of climate change.

Microbial biotechnology has received a lot of attention for its potential to utilize the natural metabolic functions of microorganisms to sequester carbon. Microalgae in particular can reduce air pollution and mitigate climate change through their sequestration of CO₂ (Kumar *et al.*, 2010b). Algal biotechnology holds great promise. Algae grow well in wastewater, do not take up valuable farmland, can be seeded continuously from old cultures, have high growth rates, and their molecular constituents (carbohydrates and lipids) are valuable precursors to crude oil, nutrient supplements including power antioxidants, fertilizers, and other industrial products (Ho *et al.*, 2011; Cheah *et al.*, 2015; Even *et al.*, 2022; Barbosa *et al.*, 2023). These benefits depend on supplying the algae with CO₂ for photosynthesis; fortunately, this CO₂ can come from flue gases that would otherwise be harmful by contributing to global warming. Despite the proposed advantages of carbon capture using microalgae, there are still important issues to be addressed such as how to obtain enough microalgal biomass to make the process truly effective, sustainable and economically viable (White *et al.*, 2013; Barbosa *et al.*, 2023). Increased microalgal biomass

can be achieved through co-culture of microalgae with bacteria that improve both growth and physiology of the microalgae (Palacios *et al.*, 2022). The symbiosis of bacteria and microalgae can improve carbon capture systems as increased algal growth and lipid production result in more carbon molecules stored and utilized. In order to produce algal cultures with enough biomass and lipid content to produce biofuels that are economically competitive, equal attention must be paid to both algae and their bacterial symbionts (Tong *et al.*, 2023). One research objective at the start of this dissertation was to analyze the bacterial communities in the context of carbon capture functionality and the productivity of the overall system. While this objective continues to be a goal of the Hill laboratory (discussed in Section 6.3.3), the detection of two bacteria belonging to previously unclassified bacterial lineages was unexpected and became the focus of my work. The first goal of my dissertation was to complete a milestone set forth in our research funded by the US Department of Energy (DOE) to conduct 16S rRNA gene sequencing on all relevant microalgal cultures across various experiments to characterize bacterial communities and identify dominant taxa. After understanding the taxonomic composition, I continue to target dominant organisms to uncover whether they may be contributing positively or negatively to microalgal growth and culture stability. What I found was dominance by bacteria that were only assigned the name “uncultured bacterium clone”. While finding uncultured bacteria (often labeled as “Other”) in microbiome datasets is normal, seeing them as the most abundant bacteria was surprising. Attempting to recover their genomes from metagenomic sequence data became the priority of my doctoral work with the aim of uncovering the mystery of their taxonomy and their function. I found that two bacteria, *Phycocordibacter aenigmaticus* and *Minuscolum obligatum*, do not encode for any carbon cycling genes (Figure 5.2), but they

have the potential to indirectly aid in carbon capture through their production of plant vitamins and other plant-growth promoting traits (Figure 5.5).

6.2 Zooming in on both microalgal cultures: Striking and unexpected dominance of two novel Patescibacteria

The dominance of Patescibacteria in cultures of both *N. oceanica* and *T. obliquus* allowed for me to gain insights into this phylum that are normally unattainable. Both *M. obligatum* and *P. aenigmaticus* were found to be associated with two microalgae at two salinities—the freshwater strain, *T. obliquus* HTB1 and the marine strain, *N. oceanica* IMET1—at two different scales (1 liter and 500 liters), within two different filter size fractions (0.45 μm and 0.22 μm), and regardless of whether cultures were bubbled with ambient air, 5% CO_2 , or 10% CO_2 (Table 6.1). They were found more often and at higher relative abundance in *N. oceanica* samples, and no Patescibacteria were found in the 6,800 L bioreactor (Table 6.1 and Figure 3.11 | Chapter 3). When *N. oceanica* was bubbled with 10% CO_2 at the 1 L scale, *M. obligatum* and *P. aenigmaticus* made up 79.7% relative abundance. In the 500 L bioreactors with 5% CO_2 , *M. obligatum* and *P. aenigmaticus* were also dominant, making up >50% relative abundance in *T. obliquus* cultures and >95% relative abundance in *N. oceanica* cultures. However, the dominance of *M. obligatum* and *P. aenigmaticus* did not remain stable over the course of my research. I was unable to find either bacterium when sequencing various *N. oceanica* culture a year and a half later (Figure 3.12, Table 3.3 | Chapter 3). I did find another related taxon “Parcubacteria group bacterium” in one sample (Figure 3.12) although it was at <1% relative abundance. This result

indicates that more Patescibacteria may be within our microalgal system and could perhaps become dominant under certain environmental conditions.

The very high relative abundance of these two bacteria made it possible to recover complete and high-quality metagenomes. This allowed me to conduct a complete metabolic reconstruction of *M. obligatum* and *P. aenigmaticus*. Similar to other Patescibacteria, neither bacterium has complete pathways in central carbohydrate metabolism, ATP synthesis, or amino acid biosynthesis. They are most likely obligate symbionts of the microalgae or neighboring bacteria, which could explain why both are yet unable to be cultivated outside of our algal system. For nutrient cycling genes, no complete pathway was encoded within either bacterium. There were partial pathways for denitrification and sulfur oxidization encoded by *P. aenigmaticus*, but they are likely insufficient to support metabolism. They may contribute to inorganic nutrient cycling or shared redox reactions within the phycosphere. As we enter an era in which thousands of MAGs are able to be analyzed at once (Anantharaman *et al.*, 2016), metabolic inferences across various members of Patescibacteria will contribute knowledge to a rapidly advancing body of research on the scavenging metabolic nature of this enigmatic and largely unclassified phylum.

Table 6.1. Samples in which *Minuscolum obligatum* and *Phycocordibacter aenigmaticus* were found.

Microalgae	Scale	Air or CO ₂	Novel Patescibacteria, <i>M. obligatum</i> and <i>P. aenigmaticus</i> found?
<i>N. oceanica</i>	1 L	10% CO ₂	Yes
<i>N. oceanica</i>	1 L	Air	Yes
<i>N. oceanica</i>	500 L	5% CO ₂	Yes
<i>T. obliquus</i>	1 L	10% CO ₂	No
<i>T. obliquus</i>	1 L	Air	No
<i>T. obliquus</i>	500 L	5% CO ₂	Yes
<i>T. obliquus</i>	6,800 L	5% CO ₂	No

6.3 Limitations and future directions

6.3.1 Limitations of working with uncultured Patescibacteria

One major limitation to working with Patescibacteria is that most are unable to be cultivated in the laboratory. The few that have been cultured are obligate symbionts that attach to host cell surfaces (He *et al.*, 2015; Batinovic *et al.*, 2021; Yakimov *et al.*, 2022; Wang *et al.*, 2023b). Because we are unable to grow Patescibacteria in pure culture, their metabolism cannot be directly tested in an experimental setting. For example, claims of amino acid auxotrophy in this dissertation are based on genes involved in biosynthesis pathways and tRNAs. If *M. obligatum* and *P. aenigmaticus* could be grown in culture, I could develop various media with certain missing amino acids and test survival. This could be especially interesting if I was able to obtain a culture of *M. obligatum* or *P. aenigmaticus* along with a microalgal host. By developing media

missing specific amino acids or carbon compounds, I could directly test whether these compounds are supplied by the microalgae.

In this work, I can only speculate on functional contributions of bacteria with the microalgal system. In order to make valid claims of bacterial input, one would need to conduct a multilayered transcriptomics/proteomics/metabolomics framework to elucidate gene expression and protein/metabolite composition. Further, confirming the presence of Patescibacteria in a sample can only be validated in a process that is costly and takes weeks for a result; one must extract DNA and conduct 16S rRNA sequencing (\$100 per sample at this time) and bioinformatically analyze the entire microbiome. One could instead design targeting PCR primers, but doing so would require having a genome or a full length 16S rRNA sequence. In the case of this research, I have obtained high quality genomes and could target multiple genetic regions when experimenting with primer design. Once specific and reliable primers were established, one could test samples continuously for the presence of *P. aenigmaticus* and *M. obligatum* using PCR. These primers could also be amended with a probe and used for FISH or flowcytometry. Considering bacterial communities frequently shift over time, between treatments, and between microalgal seed cultures, this process would need to be repeated continuously to track whether the Patescibacteria of interest were still present.

6.3.2 Limitations and considerations when working within a highly unclassified lineage

Researchers working with Patescibacteria are currently at an exciting frontier, although working with a phylum in which the majority of lower taxonomic ranks remain unnamed can pose challenges (Srinivas *et al.*, 2024). Both *M. obligatum* and *P. aenigmaticus* have no closely

related MAGs that have been formally named and published (Figure 4.6 | Chapter 4), even at the order level (Figure 4.17B). The nearest cultured relatives are *Saccharimonas aalborgensis* and *Cand. Absconditicoccus praedator* (Figure 4.17C), and both belong to different classes than that of *M. obligatum* and *P. aenigmaticus* (Yakimov *et al.*, 2022). This fact can make 16S rRNA gene marker trees (Figures 4.3, 4.5, and 4.6) and concatenated gene phylogenomic trees (Figure 4.17) not as informative as trees that display more highly classified lineages. As more classes, orders, and lower ranks of Patescibacteria are designated as type strains and given formal names, both gene marker and concatenated gene trees will become more informative. In this time during which most members of Patescibacteria have alphanumeric codes instead of names, it is recommended to construct a phylogeny with 120 ubiquitous single-copy marker genes (Figure 4.17), use continuously updated (and publicly available) taxonomic resources such as the TYGS and GTDB, and propose names with collaboration with the SeqCode (Parks *et al.*, 2018b, 2022; Meier-Kolthoff *et al.*, 2022; Riesco and Trujillo, 2024; Srinivas *et al.*, 2024).

6.3.3 Future Directions

This dissertation raises two important research questions for further exploration. The first is: “how can bacterial symbionts be targeted for improving carbon capture systems?” One could manipulate microbiomes or target specific bacterial taxa with the aim of directly enhancing algal growth. One question that arose during my microscopy was whether I was able to image the natural spatial associations of bacteria and microalgae. For SEM, samples have to undergo several rounds of washing and centrifugation, for example in the fixation steps. When imaging microalgae/bacteria associations again, Cryo-EM could be a promising methodology and has been conducted previously on microalgae (Wan *et al.*, 2024). Cryo-EM does not require that sample first be dehydrated, which is advantageous as various details may be lost due to damage

caused by dehydration (Wan et al., 2024). The first method in Cryo-EM is rapidly freezing samples, thereby keeping natural cell associations intact.

In order to understand which bacteria may be of interest, it is advantageous to first consider total bacterial DNA within the system. This is accomplished by metagenomic sequencing and allows for researchers to quickly obtain a holistic snapshot of the system. Further, conducting a functional gene analysis on total reads and constructing metagenomes to which the functions can be assigned allows for researchers to highlight which bacterial symbionts encode for advantageous functions. Metagenomic sequencing leading to MAG recovery and functional gene analysis provides a necessary framework for future steps. The next rational step would be total RNA sequencing and transcriptomic analysis. As transcriptomes only provide a singular snapshot of which mRNA transcripts are present at the time of sampling, experiments must be designed so that multiple samples are taken between treatments (*e.g.* samples bubbled with CO₂ vs Air) and across time scales. When working with unclassified organisms such as *Patescibacteria*, transcriptomic data is only valuable with high quality MAGs as these organisms are often underrepresented or absent from public databases. Transcriptomic datasets can be supplemented further with protein (proteomic) analysis and metabolite (metabolomic) analysis. Similar to transcriptomes, these analyses are valid only when protein sequences can be matched directly back to specific organisms within original metagenomic datasets. While omics datasets provide an extensive look across the entire bacterial community, they are most useful if a bacterium of interest can be grown and manipulated in a laboratory setting.

To effectively enhance microalgal systems using a growth-promoting bacterium of interest, it is a huge advantage if the bacterium can be cultivated. Further, once the bacterium is

grown to a sufficient density it can be added back into the microalgal system. This work is currently being done in the Hill laboratory by Kieran Szelong using a bottom-up approach. Instead of first identifying bacteria of interest through omics work (top-down approach), several bacterial isolates are grown by plating cultures of *N. oceanica* and *T. obliquus*. These bacteria are then reinoculated back into microalgal cultures and tested for growth enhancement compared to two controls: unmanipulated microalgal cultures and microalgal cultures inoculated with *Escherichia coli*, the latter serving as a general bacterial control. While some bacteria do not stimulate enhanced growth, our laboratory has isolated six bacteria that, when reinoculated into microalgal cultures, enhance the growth of the microalgae. Growth is measured by optical density, cell counting, dry weight, ash-free dry weight, and chlorophyll-*a* content. The next steps are underway to identify the mechanisms of growth enhancement such as bacterial production of auxins, gibberellins, cytokinins, and quorum sensing molecules.

It is also conceivable that the growth promoting benefits of a bacterium could be enhanced even in cases in which the bacterium is not cultured. After a full omics analysis, the mechanism(s) of growth promotion could be deduced as could some of the growth requirements of the bacterium. The culture medium in which the microalgae and symbionts are grown could perhaps be supplemented with factor/s that promote the *in situ* growth of the bacterium even though it has never been cultured in pure culture. The appropriate media amendments may increase the relative density of the growth promoting bacterium and lead to enhanced microalgal growth.

The second major research question to explore from this dissertation is: “What is the role of the dominant *M. obligatum*, *P. aenigmaticus*, and other Patescibacterial symbionts in microalgal cultures?” As a Parcubacteria group bacterium was found in *N. oceanica* cultures

(Figure 3.12 | Chapter 3), it is likely that other Patescibacteria are associated with *N. oceanica* and *T. obliquus*. The only way to detect more Patescibacteria would be continued 16S rRNA sequencing and microbiome analysis. When they are found once more, the cultures could undergo fluorescence *in situ* hybridization (FISH) using custom Patescibacteria primers and imaging with confocal laser microscopy (Wang *et al.*, 2023b). Attempts could be made to coculture *M. obligatum* and *P. aenigmaticus* with an algal or bacterial host; however, only four members of the entire phylum (all from human saliva) have been cultured thus far (He *et al.*, 2015; Batinovic *et al.*, 2021; Yakimov *et al.*, 2022; Wang *et al.*, 2023b).

6.4 Overall conclusions and final takeaways

Because of their ability to naturally sequester CO₂ through photosynthesis and the various value-added products that can be produced from their biomass, microalgae have emerged as promising candidates for CO₂ mitigation. Further, carbon removal using microalgae can be paired with other technologies such as wastewater treatment. Researcher now understand that microalgae do not generally grow in axenic cultures. Instead, the phycosphere contains a complex assemblage of bacteria that can positively or negatively affect microalgal growth, physiology, and survival. This dissertation used multiple techniques to determine the identity, location, and function of bacteria associated with two microalgae optimized for CO₂ sequestration, *T. obliquus* and *N. oceanica*. I conducted several rounds of epifluorescent microscopy and six rounds of Scanning Electron Microscopy (SEM) on both *N. oceanica* strain IMET1 and *T. obliquus* strain HTB1 to visualize the spatial relationship between each microalgae and their respective bacterial symbionts. Microscopy showed that both microalgal cultures contain bacterial symbionts that are closely associated (attached to the algal cell) and

free-living. I then used dPCR to quantify microalgal and bacterial cells. In *N. oceanica* cultures, the ratio of microalgal cells to bacterial cells to was 1:5 both for closely associated symbionts and for free-living symbionts. In *T. obliquus* cultures, the ratio of microalgal cells to bacterial cells to was found to be 2.5:1, with bacteria exclusively closely associated rather than free-living.

This work used two types of sequencing to study the bacterial communities associated with each microalga at the 1 L, 500 L, and 6,800 L scales. 16S rRNA sequencing revealed that dominant members of the *T. obliquus* bacterial communities were *Algoriphagus aquaeductus*, *Erythrobacter* sp., *Allorhizobium* sp., and *Lacibacter cauensis*. Dominant members of the easily characterized *N. oceanica* bacterial communities were *Gracilimonas sediminicola*, *Microcella pacifica*, and *Maricaulis maris*. Two unnamed bacteria were found in high relative abundance in both microalgal cultures, making up to 95% of the bacterial community. Six rounds of metagenomic sequencing were performed in an attempt to recover genomes for these two enigmatic bacteria as well as other bacteria within the system. Seven MAGs were successfully recovered: five previous characterized bacteria—*Gracilimonas sediminicola*, *Microcella pacifica*, *Allorhizobium* sp., *Erythrobacter* sp., and *Algoriphagus aquaeductus*—as well as the two enigmatic bacteria which I named *Minusculum obligatum* gen. nov. sp. nov. and *Phycocordibacter aenigmaticus* gen. nov. sp. nov through the Seqcode (seqco.de/r:ywe1blo2). Both belong to a novel family, *Phycocordibacteraceae* fam. nov. and a novel order, *Phycocordibacterales* ord. nov. I then conducted functional gene profiling and metabolic reconstruction to understand each bacterium's potential contributions to the microalgal system. The five well characterized MAGs encode for several genes involved in nutrient cycling, such as partial denitrification pathways as well as iron reduction. *P. aenigmaticus* and *M. obligatum* show patterns consistent with other Patescibacteria in that their genomes do not encode complete

glycolysis and TCA pathways and lack machinery to synthesize any amino acids. Instead, they encode for the reductive pentose phosphate pathway (Calvin cycle) for essential biosynthesis and redox balance. Based on their highly reduced genomes and their lifestyle prediction scores using the program, symcla, I ascertain both bacteria are highly obligate, but not endosymbiotic. There is a likely possibility that both *M. obligatum* and *P. aenigmaticus* are symbionts of a neighboring bacterium/bacteria as opposed to the microalgae, as suggested by their dominance in the free-living (0.22 μm) fraction and because I never found them solely in the algal associated (0.45 μm) fraction. Metagenomic datasets continue to grow using brand-new long read sequencing technologies, and the vast majority of DNA sequences obtained are derived from prokaryotes that are unable to be cultivated in the laboratory. Members of the enormous but vastly unclassified phylum, Patescibacteria, will continue to be uncovered with the likelihood that they play important roles in ecosystem functioning and future bioremediation strategies.

Appendix to Chapter 2

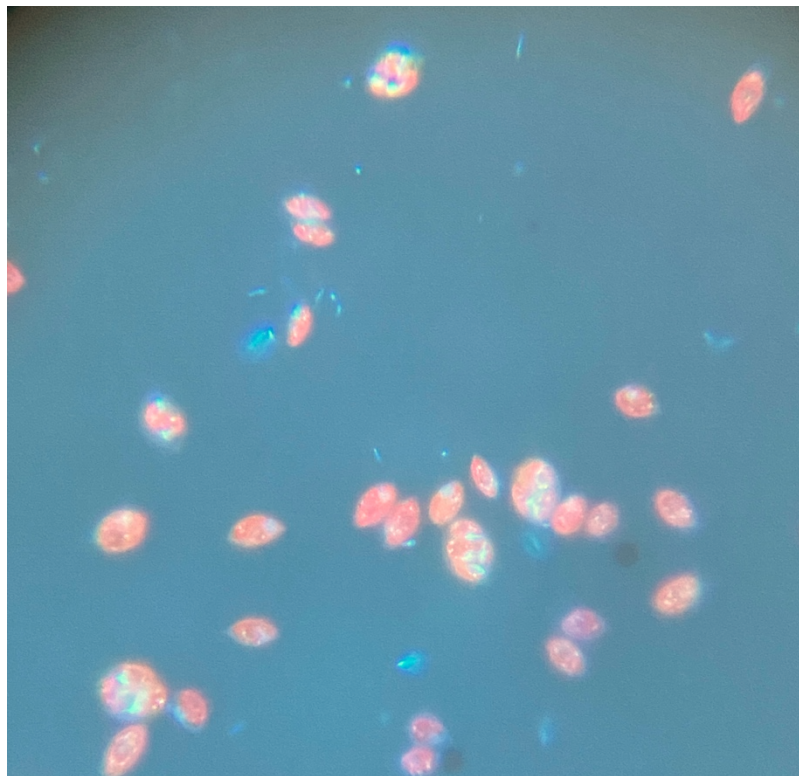


Figure A2.1 *T. obliquus* HTB1 cultures stained with DAPI and excited by laser (405 nm excitation/ 430-480 nm emission) with a Leica Confocal microscope and DAPI Long channel filter cube. Photos were taken with an iPhone 11 through the microscope eye piece.

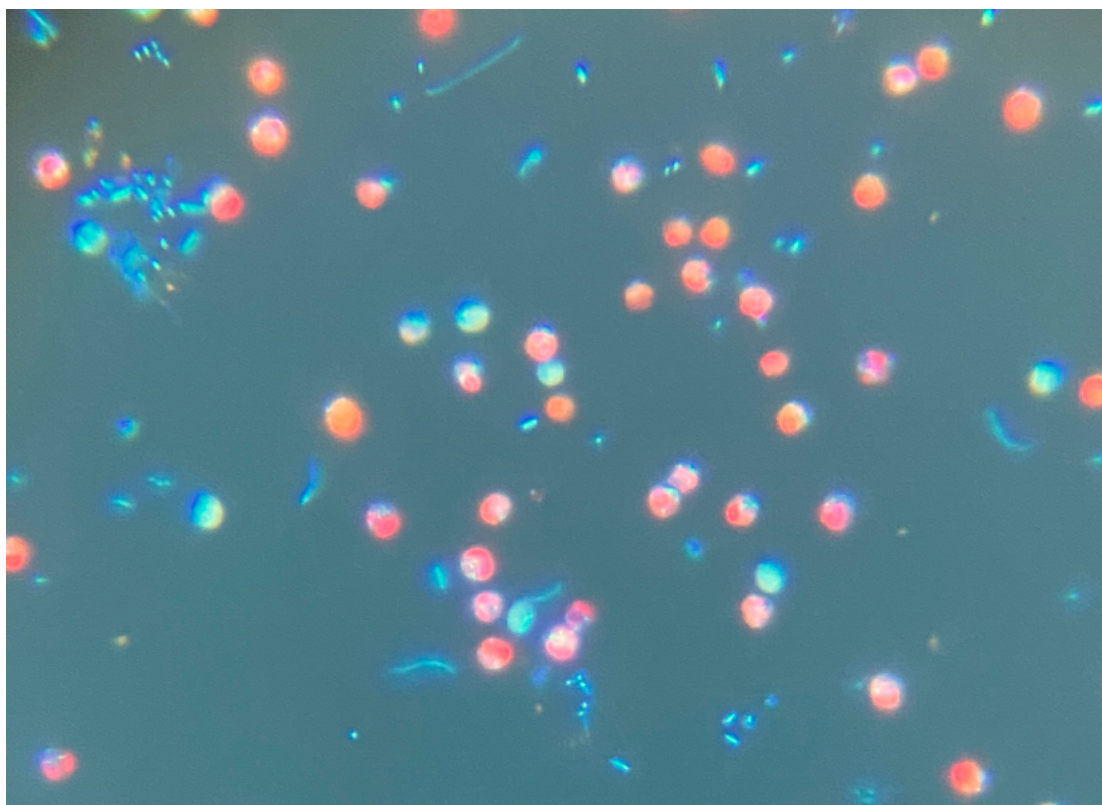
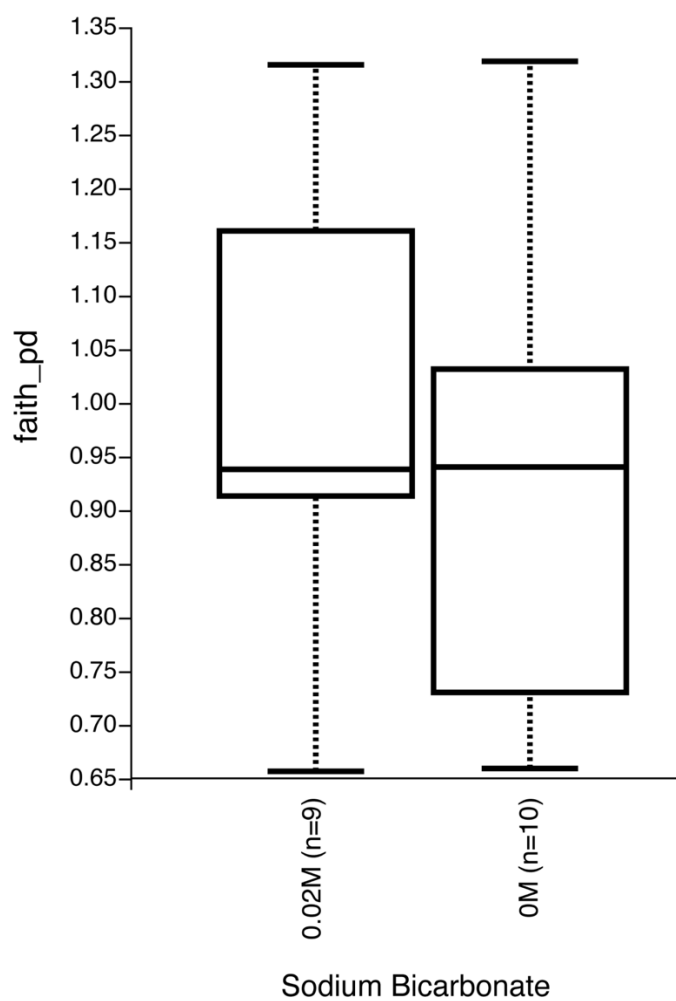


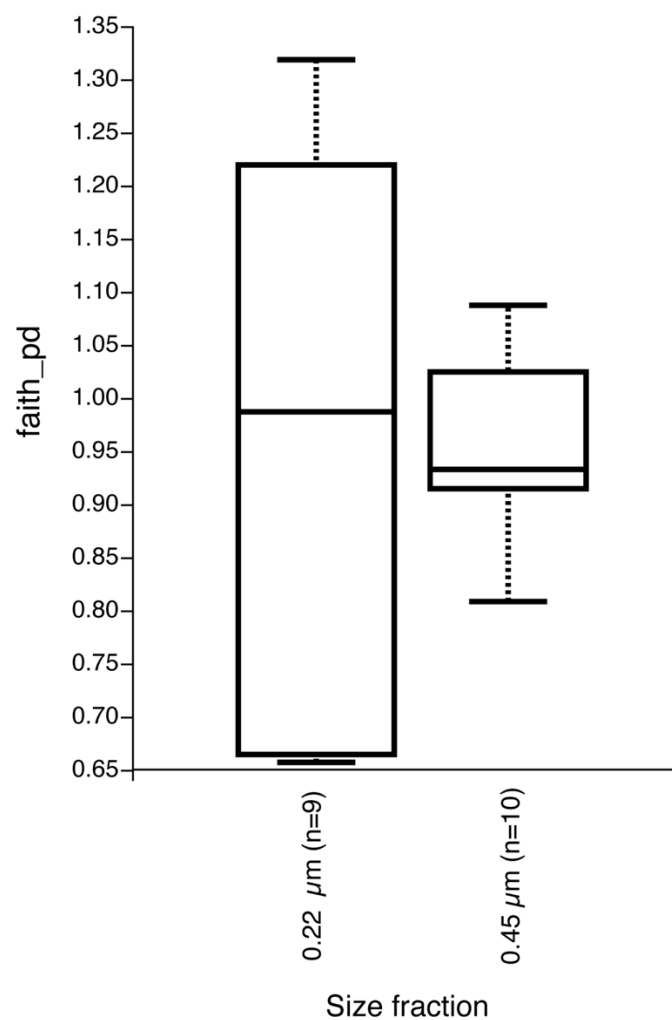
Figure A2.2 *N. oceanica* IMET1 cultures stained with DAPI and excited by laser (405 nm excitation/ 430-480 nm emission) with a Leica Confocal microscope and DAPI Long channel filter cube. Photos were taken with an iPhone 11 through the microscope eye piece.

Appendix to Chapter 3



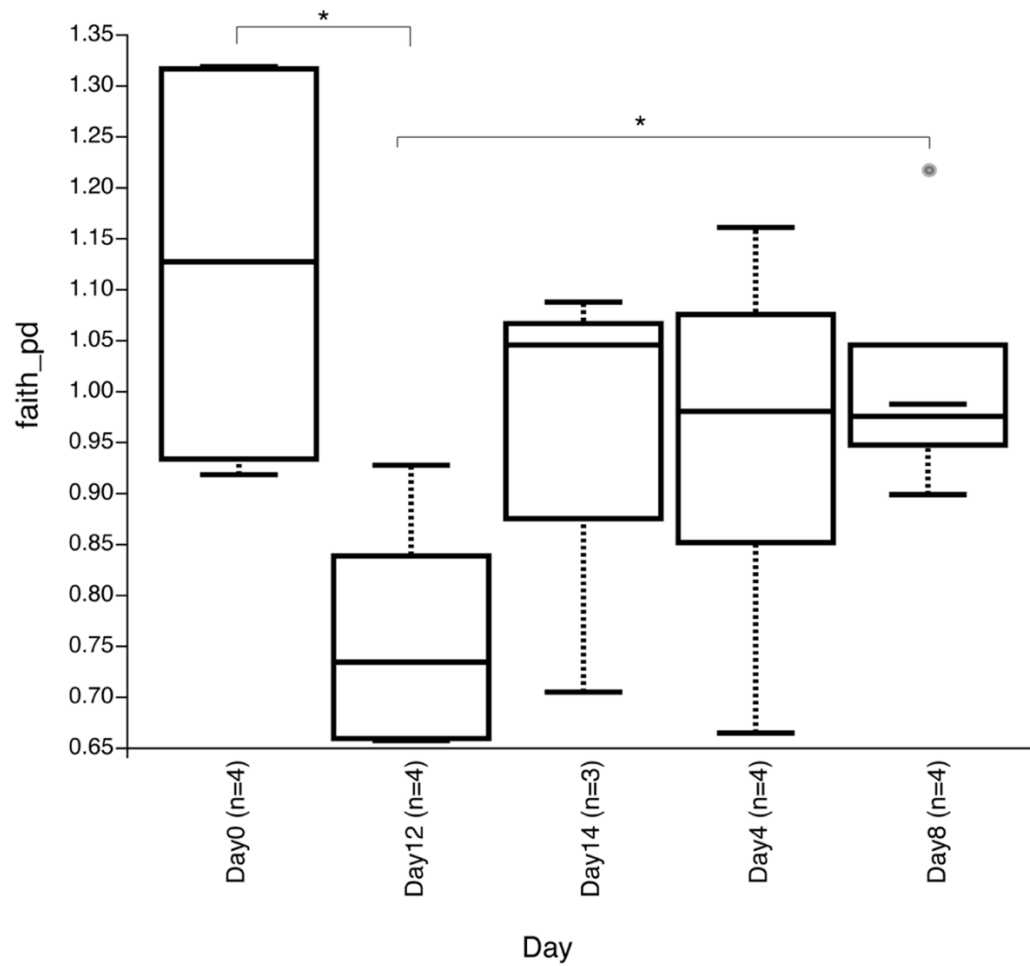
Group 1	Group 2	H	p-value	q-value
0.02M (n=9)	0M (n=10)	0.32666667	0.5676285	0.5676285

Figure A3.1 Alpha group significance using Faith's Phylogenetic comparing bacterial communities in *N. oceanica* samples receiving 10% CO₂ and no NaHCO₃ (0 M) and 0.02 M NaHCO₃. Relative abundance plots shown in Figure 3.9.



Group 1	Group 2	H	p-value	q-value
0. 22 μm (n=9)	0.45 μm (n=10)	0.02666667	0.87028277	0.87028277

Figure A3.2 Alpha group significance using Faith's Phylogenetic comparing bacterial communities in *N. oceanica* samples of different fraction sizes, 0.45 μm vs. 0.22 μm receiving 10% CO₂. Relative abundance plots shown in Figure 3.9.



Group 1	Group 2	H	p-value	q-value
Day0 (n=4)	Day12 (n=4)	4.08333333	0.04330814	0.21654071
Day0 (n=4)	Day14 (n=3)	0.5	0.47950012	0.79916687
Day0 (n=4)	Day4 (n=4)	1.33333333	0.24821308	0.49642616
Day0 (n=4)	Day8 (n=4)	0.33333333	0.56370286	0.8052898
Day12 (n=4)	Day14 (n=3)	2	0.15729921	0.39324802
Day12 (n=4)	Day4 (n=4)	2.08333333	0.14891467	0.39324802
Day12 (n=4)	Day8 (n=4)	4.08333333	0.04330814	0.21654071
Day14 (n=3)	Day4 (n=4)	0	1	1
Day14 (n=3)	Day8 (n=4)	0	1	1
Day4 (n=4)	Day8 (n=4)	0.08333333	0.77282999	0.96603749

Figure A3.3 Alpha group significance using Faith's Phylogenetic comparing bacterial communities in *N. oceanica* samples receiving 10% CO₂ across timepoints (Day 0, 4, 8, 12, 14).

Relative abundance plots shown in Figure 3.9.

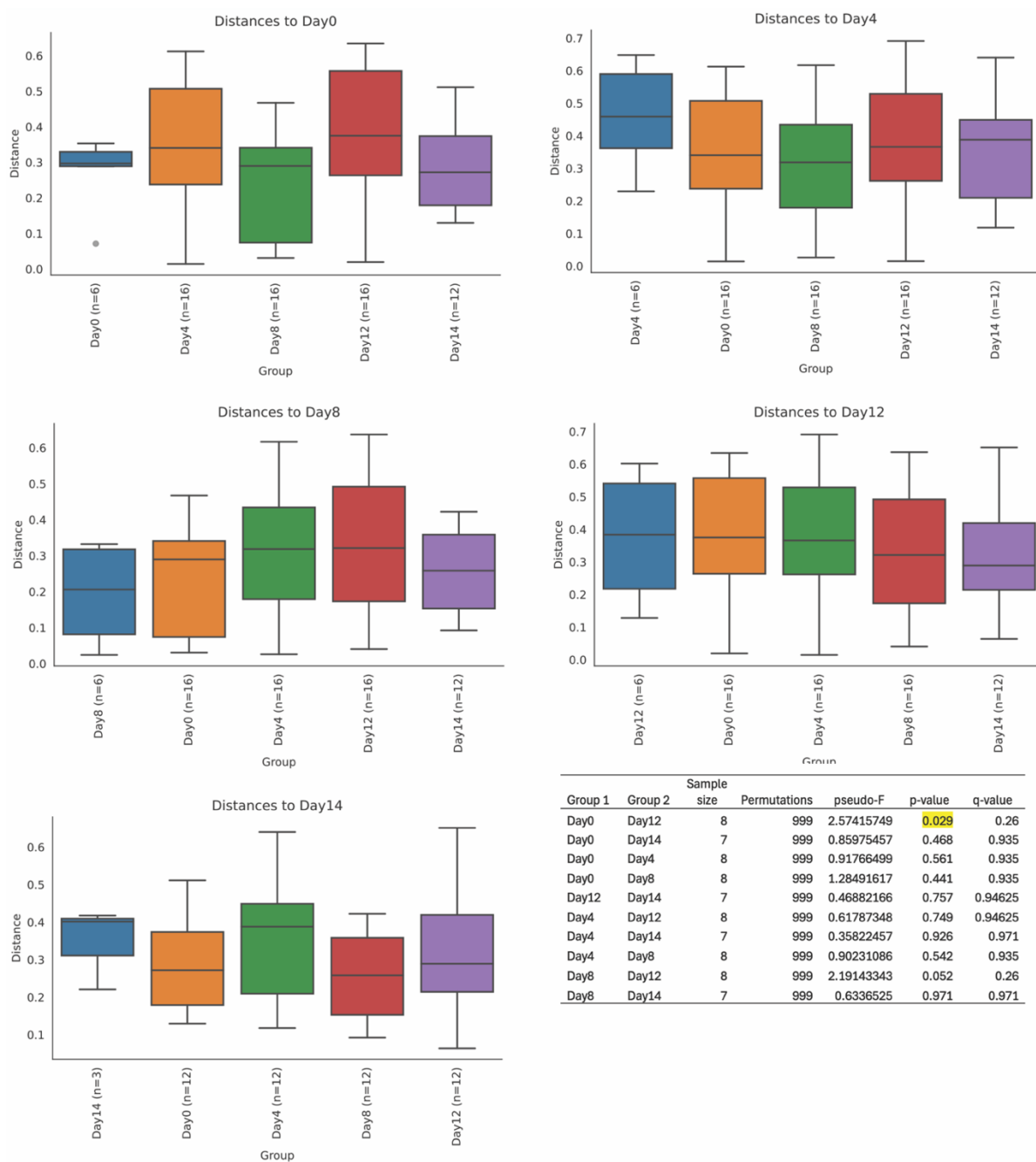


Figure A3.4 Beta diversity analyzed with Unifrac distances (unweighted) comparing bacterial communities in *N. oceanica* samples receiving 10% CO₂ across timepoints (Day 0, 4, 8, 12, 14). Relative abundance plots shown in Figure 3.9.

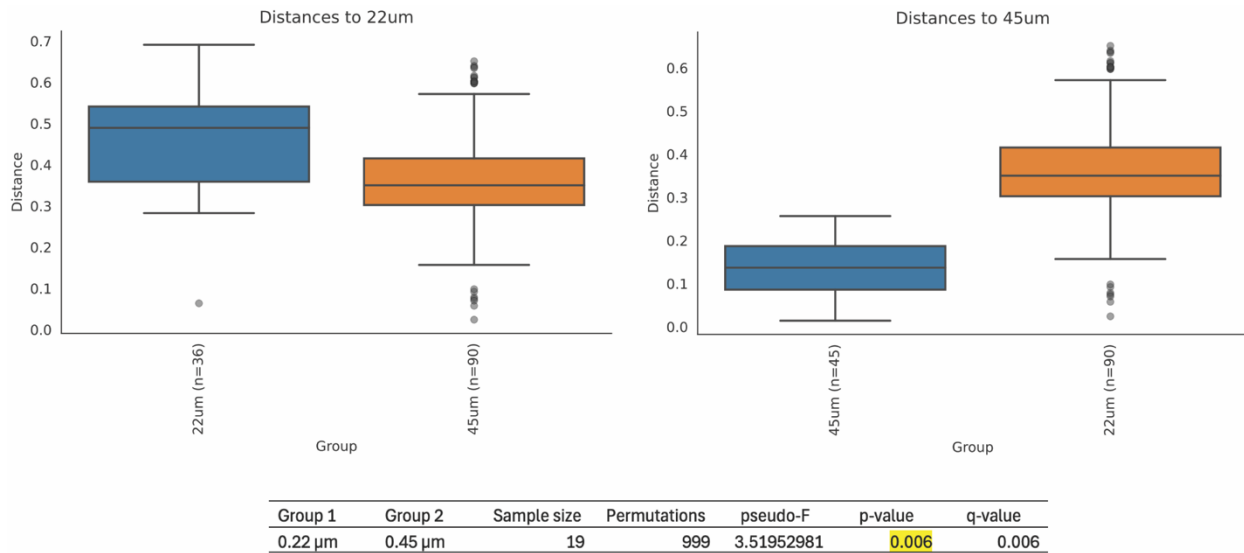


Figure A3.5 Beta diversity analyzed with Unifrac distances (unweighted) comparing bacterial communities in *N. oceanica* samples receiving 10% CO₂ of different fraction sizes, 0.45 μm vs. 0.22 μm. Relative abundance plots shown in Figure 3.9.

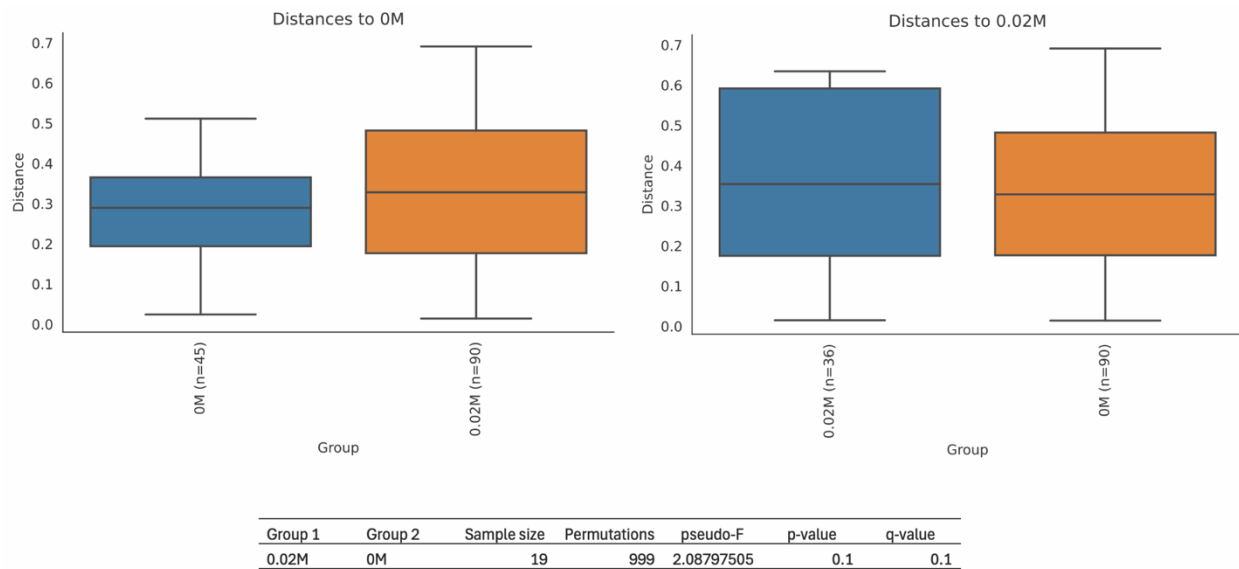


Figure A3.6 Beta diversity analyzed with Unifrac distances (unweighted) comparing bacterial communities in *N. oceanica* samples receiving 10% CO₂ receiving no NaHCO₃ (0 M) and 0.02 M NaHCO₃. Relative abundance plots shown in Figure 3.9.

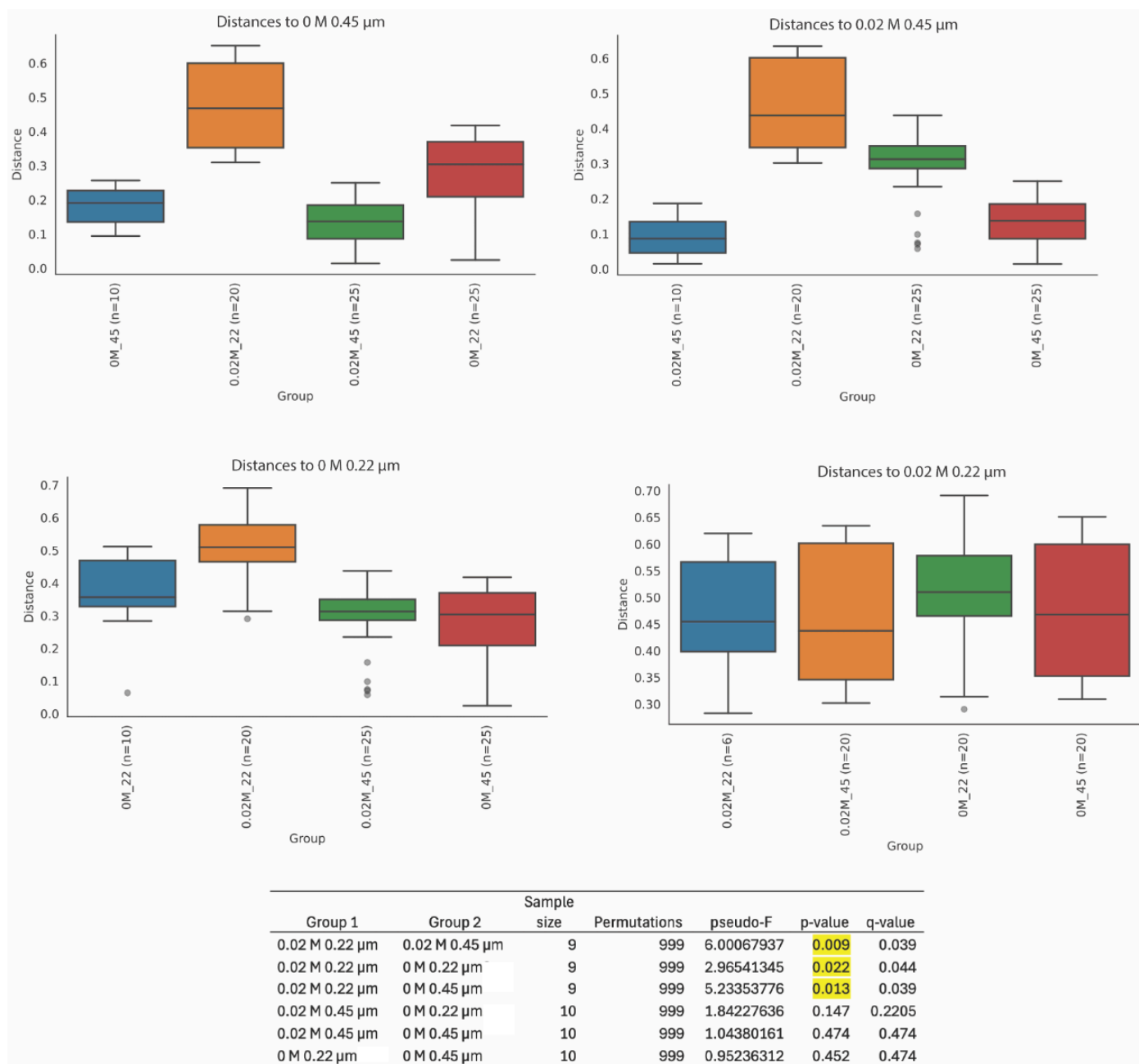


Figure A3.7 Beta diversity analyzed with Unifrac distances (unweighted) comparing bacterial communities in 0.45 μm -fractionated *N. oceanica* samples receiving 10% CO_2 supplemented with 0 M and 0.02 M NaHCO_3 and 0.22 μm -fractionated samples supplemented with 0 M and 0.02 M NaHCO_3 . Relative abundance plots shown in Figure 3.9.

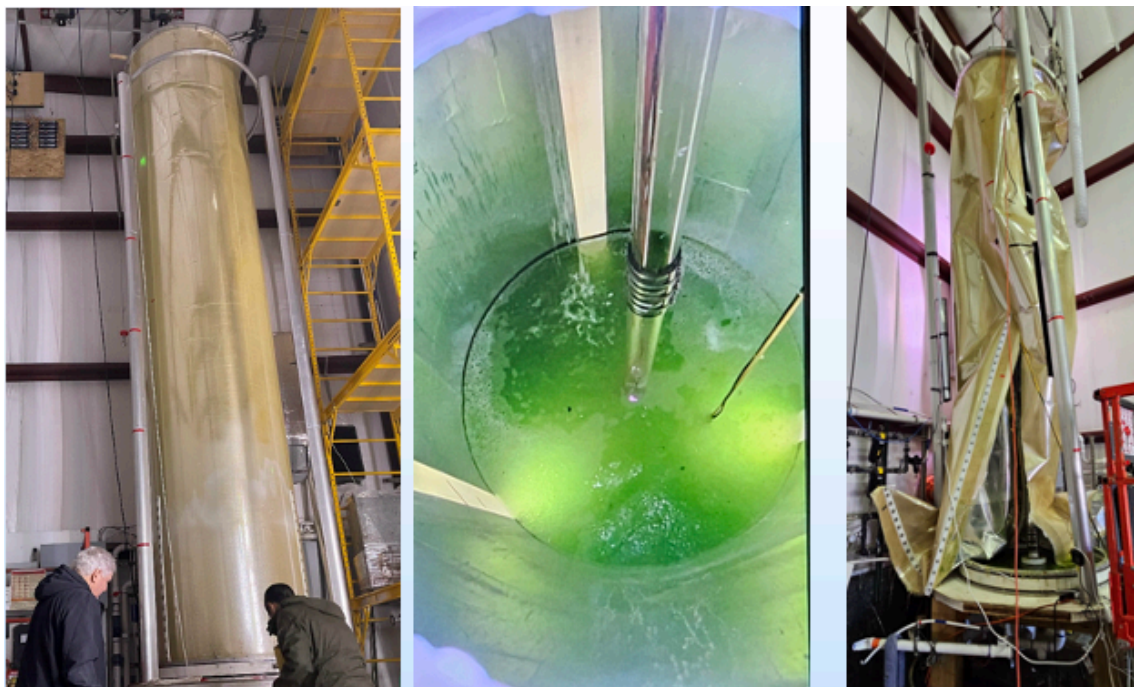


Figure A3.8 Photos of the 6,800 L bioreactor at HyTek Bio before collapse (left photo), containing *T. obliquus* (middle photo), and after collapse (right photo).

Appendix to Chapter 4

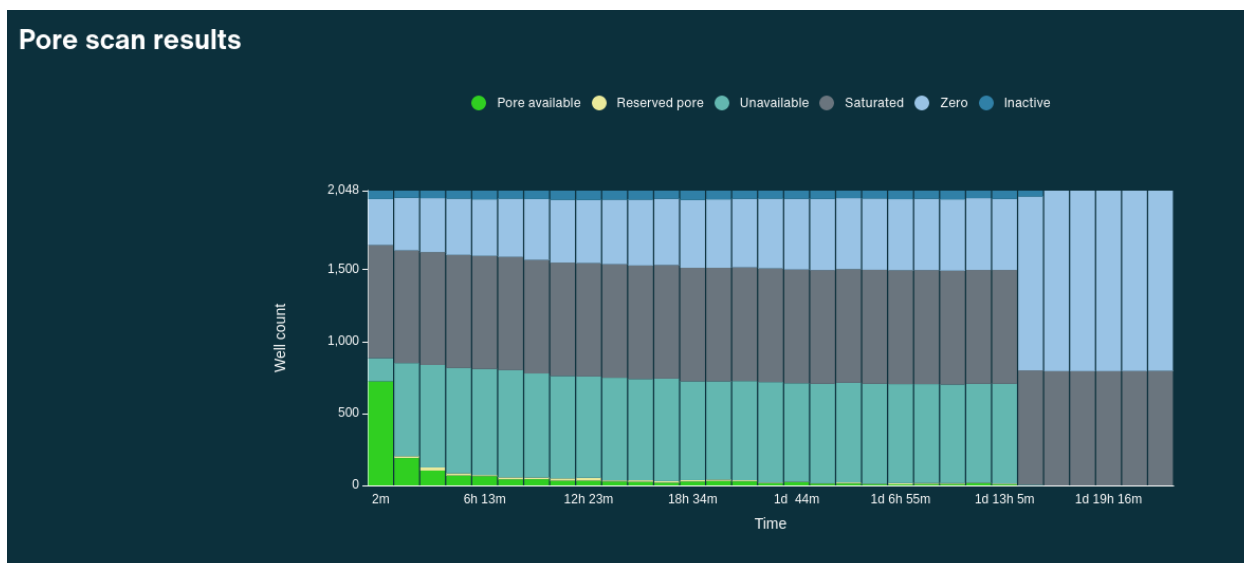


Figure A4.1 Output displaying pores available with the Nanopore chip sequencer during sequencing round, MAG4.

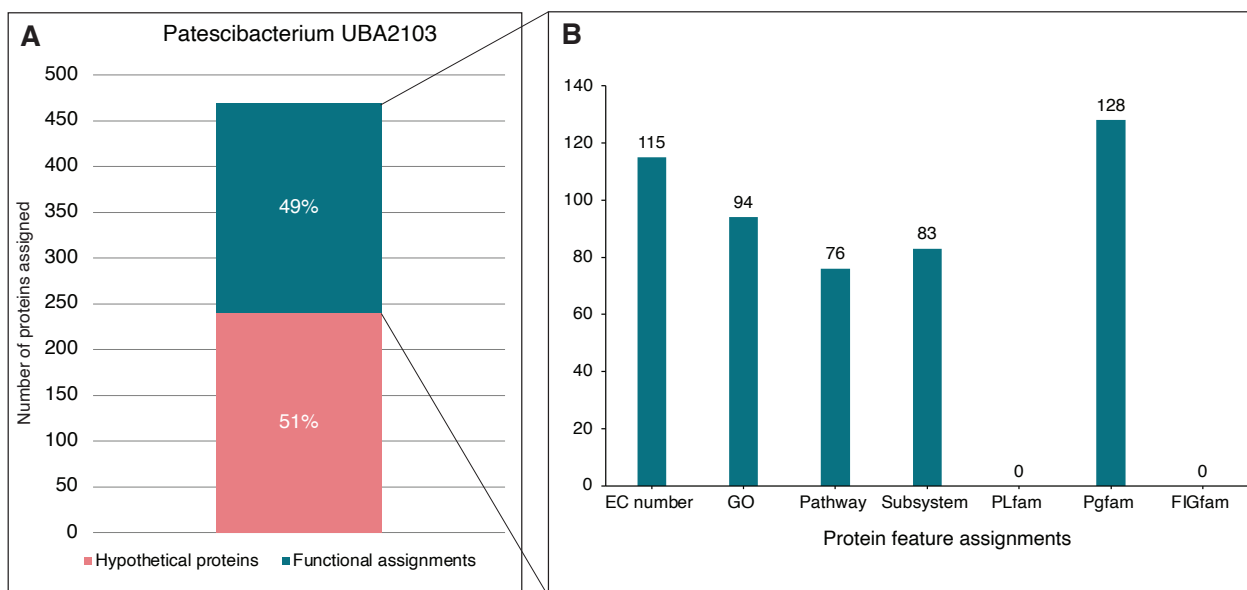


Figure A4.2 Assignment of protein features in *Minusculum obligatum* (Patescibacterium UBA2103) by the Pathosystems Resource Integration Center (PATRIC), now provided through

Bacterial and Viral Bioinformatics Resource Center (BV-BRC). (A) Percentages of total proteins designated with functional assignments (teal) and hypothetical assignments (pink). (B) Number of protein feature assignments within various databases: EC: Enzyme Commission, GO: Gene Ontology, PLfam: PATRIC genus-specific protein families, PGFam: PATRIC global protein families, FIGfam: custom manually annotated proteins.

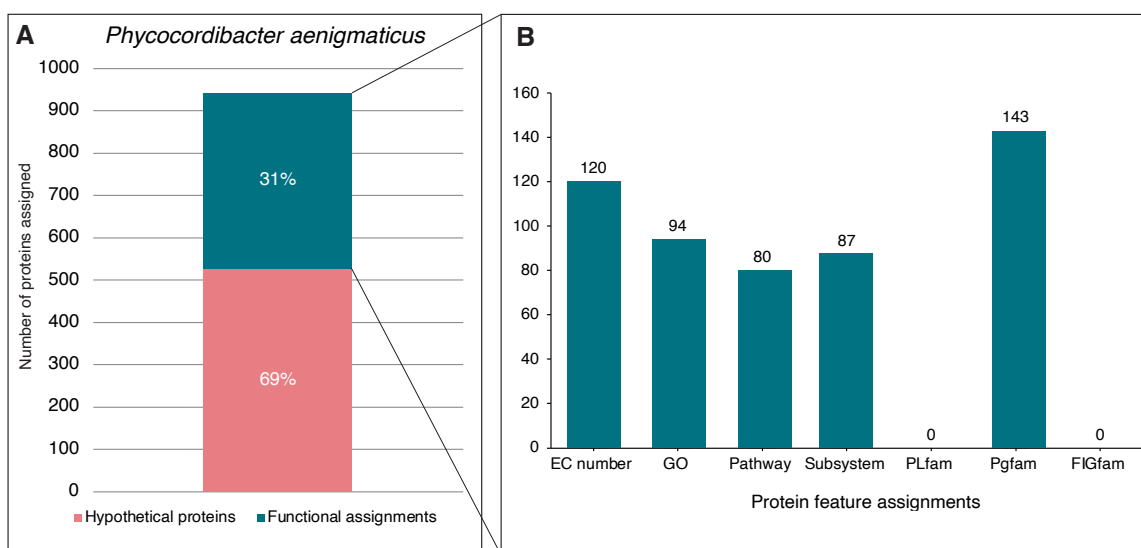


Figure A4.3. Assignment of protein features in *Phycocordibacter aenigmaticus* by the Pathosystems Resource Integration Center (PATRIC), now provided through Bacterial and Viral Bioinformatics Resource Center (BV-BRC). (A) Percentages of total proteins designated with functional assignments (teal) and hypothetical assignments (pink). (B) Number of protein feature assignments within various databases: EC: Enzyme Commission, GO: Gene Ontology, PLfam: PATRIC genus-specific protein families, PGFam: PATRIC global protein families, FIGfam: custom manually annotated proteins.

#seq_name	length	cov.	circ.	repeat	mult.	alt_group	graph_path
contig_125	2778058	75	N	N	1	*	160,125,160
contig_511	2780036	85	N	N	1	*	514,511,510,514

Figure A4.4 Output of assembly_info.fasta from Flye assembly algorithm (v 2.9.3) showing the major contigs of *Microcella pacifica*. Contig_125 was recovered from sequencing round, MAG1_Round1, in June 2023 and contig_511 was recovered from sequencing round, MAG3, in April 2024.

Appendix to Chapter 5

Pathway	Steps
<u>arg</u>	<u>argA</u> , <u>argB</u> , <u>argC</u> , <u>argD'B</u> , <u>argF'B</u> , <u>argE'B</u> , <u>argG</u> , <u>argH</u>
asn	aspS2 , gatA , gatB , gatC
<u>chorismate</u>	<u>aroG</u> , <u>aroB</u> , <u>aroD</u> , <u>aroE</u> , <u>aroL</u> , <u>aroA</u> , <u>aroC</u>
<u>cys</u>	<u>cysE</u> , <u>cysK</u>
<u>gln</u>	<u>gltX</u> , <u>glnA</u> , gatA , gatB , gatC
gly	glyA
<u>his</u>	<u>prs</u> , <u>hisG</u> , <u>hisI</u> , <u>hisE</u> , <u>hisA</u> , <u>hisF</u> , <u>hisH</u> , <u>hisB</u> , <u>hisC</u> , <u>hisN</u> , <u>hisD</u>
<u>ile</u>	<u>ilvA</u> , <u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>
<u>leu</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>leuA</u> , <u>leuC</u> , <u>leuD</u> , <u>leuB</u> , <u>ilvE</u>
<u>lys</u>	<u>asp-kinase</u> , <u>asd</u> , <u>dapA</u> , <u>dapB</u> , <u>ddh</u> , <u>lysA</u>
<u>met</u>	<u>asp-kinase</u> , <u>asd</u> , <u>asd-S-transferase</u> , <u>asd-S-ferredoxin</u> , <u>asd-S-perS</u> , <u>metE</u>
<u>phe</u>	<u>cmutase</u> , <u>ptransferase</u> , <u>aro-dehydratase</u>
<u>pro</u>	<u>proB</u> , <u>proA</u> , <u>proC</u>
<u>ser</u>	<u>serA</u> , <u>serC</u> , <u>serB</u>
<u>thr</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>thrB</u> , <u>thrC</u>
<u>trp</u>	<u>trpED</u> , <u>trpD 2</u> , <u>PRAI</u> , <u>IGPS</u> , <u>trpA</u> , <u>trpB</u>
<u>tyr</u>	<u>PAH</u>
<u>val</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>

Figure A5.1 Prediction of amino acid biosynthesis in *Minuscolum obligatum* using GapMind and the Uniprot identifiers. High confidence gene predictions are in green, medium confidence in black, and low confidence in red.

Pathway	Steps
<u>arg</u>	<u>argA</u> , <u>argB</u> , <u>argC</u> , <u>argD'B</u> , <u>argF'B</u> , <u>argE'B</u> , <u>argG</u> , <u>argH</u>
<u>asn</u>	<u>aspS2</u> , <u>gatA</u> , <u>gatB</u> , <u>gatC</u>
<u>chorismate</u>	<u>aroG</u> , <u>aroB</u> , <u>aroD</u> , <u>aroE</u> , <u>aroL</u> , <u>aroA</u> , <u>aroC</u>
<u>cys</u>	<u>cysE</u> , <u>cysK</u>
<u>gln</u>	<u>gltX</u> , <u>glnA</u> , <u>gatA</u> , <u>gatB</u> , <u>gatC</u>
<u>gly</u>	<u>glyA</u>
<u>his</u>	<u>prs</u> , <u>hisG</u> , <u>hisI</u> , <u>hisE</u> , <u>hisA</u> , <u>hisF</u> , <u>hisH</u> , <u>hisB</u> , <u>hisC</u> , <u>hisN</u> , <u>hisD</u>
<u>ile</u>	<u>ilvA</u> , <u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>
<u>leu</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>leuA</u> , <u>leuC</u> , <u>leuD</u> , <u>leuB</u> , <u>ilvE</u>
<u>lys</u>	<u>asp-kinase</u> , <u>asd</u> , <u>dapA</u> , <u>dapB</u> , <u>ddh</u> , <u>lysA</u>
<u>met</u>	<u>asp-kinase</u> , <u>asd</u> , <u>asd-S-transferase</u> , <u>asd-S-ferredoxin</u> , <u>asd-S-perS</u> , <u>metE</u>
<u>phe</u>	<u>cmutase</u> , <u>ptransferase</u> , <u>aro-dehydratase</u>
<u>pro</u>	<u>proB</u> , <u>proA</u> , <u>proC</u>
<u>ser</u>	<u>serA</u> , <u>serC</u> , <u>serB</u>
<u>thr</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>thrB</u> , <u>thrC</u>
<u>trp</u>	<u>trpED</u> , <u>trpD 2</u> , <u>PRAI</u> , <u>IGPS</u> , <u>trpA</u> , <u>trpB</u>
<u>tyr</u>	<u>PAH</u>
<u>val</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>

Figure A5.2 Prediction of amino acid biosynthesis in *Phycocordibacter aenigmaticus* using GapMind and the Uniprot identifiers. High confidence gene predictions are in green, medium confidence in black, and low confidence in red.

Pathway	Steps
<u>arg</u>	<u>argJ</u> , <u>argB</u> , <u>argC</u> , <u>argD</u> , <u>carA</u> , <u>carB</u> , <u>argI</u> , <u>argG</u> , <u>argH</u>
<u>asn</u>	<u>aspS2</u> , <u>gatA</u> *, <u>gatB</u> , <u>gatC</u>
<u>chorismate</u>	<u>aroG</u> *, <u>aroB</u> , <u>aroD</u> , <u>aroE</u> , <u>aroL</u> , <u>aroA</u> , <u>aroC</u>
<u>cys</u>	<u>cysE</u> , <u>cysK</u>
<u>gln</u>	<u>glnA</u>
<u>gly</u>	<u>glyA</u>
<u>his</u>	<u>prs</u> , <u>hisG</u> , <u>hisI</u> , <u>hisE</u> , <u>hisA</u> , <u>hisF</u> , <u>hisH</u> , <u>hisB</u> , <u>hisC</u> , <u>hisN</u> , <u>hisD</u>
<u>ile</u>	<u>cimA</u> , <u>leuC</u> , <u>leuD</u> , <u>leuB</u> , <u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>
<u>leu</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>leuA</u> , <u>leuC</u> , <u>leuD</u> , <u>leuB</u> , <u>ilvE</u>
<u>lys</u>	<u>asp-kinase</u> , <u>asd</u> , <u>dapA</u> , <u>dapB</u> , <u>dapD</u> , <u>dapC</u> , <u>dapE</u> , <u>dapF</u> , <u>lysA</u>
<u>met</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>metX</u> , <u>metY</u> , <u>metH</u> *, <u>B12-reactivation-domain</u>
<u>phe</u>	<u>cmutase</u> , <u>preph-dehydratase</u> , <u>PPYAT</u>
<u>pro</u>	<u>argJ</u> , <u>argB</u> , <u>argC</u> , <u>argD</u> , <u>cyclodeaminase</u>
<u>ser</u>	<u>serA</u> , <u>serC</u> , <u>serB</u>
<u>thr</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>thrB</u> , <u>thrC</u>
<u>trp</u>	<u>trpED</u> , <u>trpD 2</u> , <u>PRAI</u> , <u>IGPS</u> , <u>trpA</u> , <u>trpB</u>
<u>tyr</u>	<u>cmutase</u> , <u>pre-dehydr</u> , <u>tyrB</u>
<u>val</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>

Figure A5.3 Prediction of amino acid biosynthesis in *Algoriphagus aquaeductus* using GapMind and the Uniprot identifiers. High confidence gene predictions are in green, medium confidence in black, and low confidence in red.

Pathway	Steps
<u>arg</u>	<u>argA</u> , <u>argB</u> , <u>argC</u> , <u>argD'B</u> , <u>argF'B</u> , <u>argE'B</u> , <u>argG</u> , <u>argH</u>
<u>asn</u>	<u>asnB</u>
<u>chorismate</u>	<u>aroG</u> , <u>aroB</u> , <u>aroD</u> , <u>aroE</u> , <u>aroL</u> , <u>aroA</u> , <u>aroC</u>
<u>cys</u>	<u>cysE</u> , <u>cysK</u>
<u>gln</u>	<u>glnA</u>
<u>gly</u>	<u>glyA</u>
<u>his</u>	<u>prs</u> , <u>hisG</u> , <u>hisI</u> , <u>hisE</u> , <u>hisA</u> , <u>hisF</u> , <u>hisH</u> , <u>hisB</u> , <u>hisC</u> , <u>hisN</u> , <u>hisD</u>
<u>ile</u>	<u>cimA</u> , <u>leuC</u> , <u>leuD</u> , <u>leuB</u> , <u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD*</u> , <u>ilvE</u>
<u>leu</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD*</u> , <u>leuA</u> , <u>leuC</u> , <u>leuD</u> , <u>leuB</u> , <u>ilvE</u>
<u>lys</u>	<u>asp-kinase</u> , <u>asd</u> , <u>dapA</u> , <u>dapB</u> , <u>DAPtransferase</u> , <u>dapF</u> , <u>lysA</u>
<u>met</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>metX</u> , <u>metB</u> , <u>metC</u> , <u>metH*</u> , <u>B12-reactivation-domain</u>
<u>phe</u>	<u>cmutase</u> , <u>preph-dehydratase</u> , <u>PPYAT</u>
<u>pro</u>	<u>proB</u> , <u>proA</u> , <u>proC</u>
<u>ser</u>	<u>serA</u> , <u>serC?</u> , <u>serB</u>
<u>thr</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>thrB</u> , <u>thrC</u>
<u>trp</u>	<u>trpE</u> , <u>trpD 1</u> , <u>trpD 2</u> , <u>PRAI</u> , <u>IGPS</u> , <u>trpA</u> , <u>trpB</u>
<u>tyr</u>	<u>PAH</u>
<u>val</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD*</u> , <u>ilvE</u>

Figure A5.4 Prediction of amino acid biosynthesis in *Allorhizobium* sp. using GapMind and the Uniprot identifiers. High confidence gene predictions are in green, medium confidence in black, and low confidence in red.

Pathway	Steps
<u>arg</u>	<u>argJ</u> , <u>argB</u> , <u>argC</u> , <u>argD</u> , <u>carA</u> , <u>carB</u> , <u>argI*</u> , <u>argG</u> , <u>argH</u>
<u>asn</u>	<u>aspS2</u> , <u>gatA</u> , <u>gatB</u> , <u>gatC</u>
<u>chorismate</u>	<u>aroG</u> , <u>aroB</u> , <u>aroD</u> , <u>aroE</u> , <u>aroL</u> , <u>aroA</u> , <u>aroC</u>
<u>cys</u>	<u>cysE</u> , <u>cysK</u>
<u>gln</u>	<u>gltX</u> , <u>glnA*</u> , <u>gatA</u> , <u>gatB</u> , <u>gatC</u>
<u>gly</u>	<u>glyA</u>
<u>his</u>	<u>prs</u> , <u>hisG</u> , <u>hisI</u> , <u>hisE</u> , <u>hisA</u> , <u>hisF</u> , <u>hisH</u> , <u>hisB</u> , <u>hisC</u> , <u>hisN</u> , <u>hisD</u>
<u>ile</u>	<u>ilvA</u> , <u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>
<u>leu</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>leuA</u> , <u>leuC</u> , <u>leuD</u> , <u>leuB</u> , <u>ilvE</u>
<u>lys</u>	<u>asp-kinase</u> , <u>asd</u> , <u>dapA</u> , <u>dapB</u> , <u>dapD</u> , <u>dapC</u> , <u>dapE</u> , <u>dapF</u> , <u>lysA</u>
<u>met</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>metX</u> , <u>metY</u> , <u>metH*</u> , <u>B12-reactivation-domain</u>
<u>phe</u>	<u>cmutase</u> , <u>preph-dehydratase</u> , <u>PPYAT</u>
<u>pro</u>	<u>proB</u> , <u>proA</u> , <u>proC</u>
<u>ser</u>	<u>serA</u> , <u>serC</u> , <u>serB</u>
<u>thr</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>thrB</u> , <u>thrC</u>
<u>trp</u>	<u>trpE</u> , <u>trpD 1</u> , <u>trpD 2</u> , <u>PRAI</u> , <u>IGPS</u> , <u>trpA</u> , <u>trpB</u>
<u>tyr</u>	<u>cmutase</u> , <u>pre-dehydr</u> , <u>tyrB</u>
<u>val</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>

Figure A5.5 Prediction of amino acid biosynthesis in *Erythrobacter* sp. using GapMind and the Uniprot identifiers. High confidence gene predictions are in green, medium confidence in black, and low confidence in red.

Pathway	Steps
<u>arg</u>	<u>argA</u> , <u>argB</u> , <u>argC</u> , <u>argD'B</u> , <u>argF'B</u> , <u>argE'B</u> , <u>argG</u> , <u>argH</u>
<u>asn</u>	<u>aspS2</u> , <u>gatA</u> , <u>gatB</u> , <u>gatC</u>
<u>chorismate</u>	<u>aroG</u> , <u>aroB</u> , <u>aroD</u> , <u>aroE</u> , <u>aroL</u> , <u>aroA</u> , <u>aroC</u>
<u>cys</u>	<u>CBS</u> , <u>CGL</u>
<u>gln</u>	<u>glnA</u>
<u>gly</u>	<u>glyA</u>
<u>his</u>	<u>prs</u> , <u>hisG</u> , <u>hisI</u> , <u>hisE</u> , <u>hisA</u> , <u>hisF</u> , <u>hisH</u> , <u>hisB</u> , <u>hisC</u> , <u>hisN</u> , <u>hisD</u>
<u>ile</u>	<u>ilvA</u> , <u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>
<u>leu</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>leuA</u> , <u>leuC</u> , <u>leuD</u> , <u>leuB</u> , <u>ilvE</u>
<u>lys</u>	<u>asp-kinase</u> , <u>asd</u> , <u>dapA</u> , <u>dapB</u> , <u>dapD</u> , <u>dapC</u> , <u>dapE</u> , <u>dapF</u> , <u>lysA</u>
<u>met</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>metX</u> , <u>metY</u> , <u>metH*</u> , <u>B12-reactivation-domain</u>
<u>phe</u>	<u>cmutase</u> , <u>ptransferase</u> , <u>aro-dehydratase</u>
<u>pro</u>	<u>argA</u> , <u>argB</u> , <u>argC</u> , <u>argD</u> , <u>argE</u> , <u>OAT</u> , <u>proC</u>
<u>ser</u>	<u>serA</u> , <u>serC</u> , <u>serB</u>
<u>thr</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>homK</u> , <u>thrC</u>
<u>trp</u>	<u>trpE</u> , <u>trpD 1</u> , <u>trpD 2</u> , <u>PRAI</u> , <u>IGPS</u> , <u>trpA</u> , <u>trpB</u>
<u>tyr</u>	<u>cmutase</u> , <u>pre-dehydr</u> , <u>tyrB</u>
<u>val</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>

Figure A5.6 Prediction of amino acid biosynthesis in *Gracilimonas sediminicola* using GapMind and the Uniprot identifiers. High confidence gene predictions are in green, medium confidence in black, and low confidence in red.

Pathway	Steps
<u>arg</u>	<u>argA</u> , <u>argB</u> , <u>argC</u> , <u>argD</u> , <u>argE</u> , <u>carA</u> , <u>carB</u> *, <u>argI</u> , <u>argG</u> , <u>argH</u>
<u>asn</u>	<u>aspS2</u> , <u>gatA</u> , <u>gatB</u> , <u>gatC</u>
<u>chorismate</u>	<u>tpiA</u> , <u>fbp</u> *, <u>asp-kinase</u> , <u>asd</u> , <u>aroA</u> ', <u>aroB</u> ', <u>aroD</u> , <u>aroE</u> , <u>aroL</u> , <u>aroA</u> , <u>aroC</u>
<u>cys</u>	<u>cysE</u> , <u>cysK</u>
<u>gln</u>	<u>glnA</u>
<u>gly</u>	<u>glyA</u>
<u>his</u>	<u>prs</u> , <u>hisG</u> , <u>hisI</u> , <u>hisE</u> , <u>hisA</u> , <u>hisF</u> , <u>hisH</u> , <u>hisB</u> , <u>hisC</u> , <u>hisN</u> , <u>hisD</u>
<u>ile</u>	<u>ilvA</u> , <u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>
<u>leu</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>leuA</u> , <u>leuC</u> *, <u>leuD</u> , <u>leuB</u> , <u>ilvE</u>
<u>lys</u>	<u>asp-kinase</u> , <u>asd</u> , <u>dapA</u> , <u>dapB</u> , <u>dapD</u> , <u>dapC</u> , <u>dapE</u> , <u>dapF</u> , <u>lysA</u>
<u>met</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>metX</u> , <u>metB</u> , <u>metC</u> , <u>metE</u>
<u>phe</u>	<u>cmutase</u> , <u>preph-dehydratase</u> , <u>PPYAT</u>
<u>pro</u>	<u>proB</u> , <u>proA</u> *, <u>proC</u>
<u>ser</u>	<u>serA</u> , <u>serC</u> *, <u>serB</u>
<u>thr</u>	<u>asp-kinase</u> , <u>asd</u> , <u>hom</u> , <u>thrB</u> , <u>thrC</u>
<u>trp</u>	<u>trpE</u> , <u>trpD 1</u> , <u>trpD 2</u> , <u>PRAI</u> , <u>IGPS</u> , <u>trpA</u> , <u>trpB</u>
<u>tyr</u>	<u>cmutase</u> , <u>pre-dehydr</u> , <u>tyrB</u>
<u>val</u>	<u>ilvI</u> , <u>ilvH</u> , <u>ilvC</u> , <u>ilvD</u> , <u>ilvE</u>

Figure A5.7 Prediction of amino acid biosynthesis in *Microcella pacifica* using GapMind and the Uniprot identifiers. High confidence gene predictions are in green, medium confidence in black, and low confidence in red.

Bibliography

- Abellan-Schneyder, I., Matchado, M. S., Reitmeier, S., Sommer, A., Sewald, Z., Baumbach, J., List, M., & Neuhaus, K. (2021). Primer, pipelines, parameters: Issues in 16S rRNA gene sequencing. *mSphere*, 6(1), e01202-20. <https://doi.org/10.1128/msphere.01202-20>
- Abid, A., Saidane, F., & Hamdi, M. (2017). Feasibility of carbon dioxide sequestration by *Spongiochloris* sp. microalgae during petroleum wastewater treatment in airlift bioreactor. *Bioresource Technology*, 236, 77-84. <https://doi.org/10.1016/j.biortech.2017.03.041>
- Abraham, W. R., Strompl, C., Meyer, H., Lindholst, S., Moore, E. R. B., Christ, R., Vancanneyt, M., Tindall, B. J., Bennasar, A., Smit, J., & Tesar, M. (1999). Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. *International Journal of Systematic Bacteriology*, 49(3), 1053-1073. <https://doi.org/10.1099/00207713-49-3-1053>
- Acinas, S. G., Antón, J., & Rodríguez-Valera, F. (1999). Diversity of free-living and attached bacteria in offshore western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. *Applied and Environmental Microbiology*, 65(2), 514-522. <https://doi.org/10.1128/aem.65.2.514-522.1999>
- Adamczyk, M., Lasek, J., & Skawińska, A. (2016). CO₂ biofixation and growth kinetics of *Chlorella vulgaris* and *Nannochloropsis gaditana*. *Applied Biochemistry and Biotechnology*, 179(7), 1248-1261. <https://doi.org/10.1007/s12010-016-2062-3>
- Alneberg, J., Bjarnason, B. S., De Bruijn, I., Schirmer, M., Quick, J., Ijaz, U. Z., Lahti, L., Loman, N. J., Andersson, A. F., & Quince, C. (2014). Binning metagenomic contigs by coverage and composition. *Nature Methods*, 11(11), 1144-1146. <https://doi.org/10.1038/nmeth.3103>
- Álvarez-Díaz, P. D., Ruiz, J., Arbib, Z., Barragán, J., Garrido-Pérez, M. C., & Perales, J. A. (2015). Wastewater treatment and biodiesel production by *Scenedesmus obliquus* in a two-stage cultivation process. *Bioresource Technology*, 181, 90-96. <https://doi.org/10.1016/j.biortech.2015.01.018>
- Amavizca, E., Bashan, Y., Ryu, C. M., Farag, M. A., Bebout, B. M., & De-Bashan, L. E. (2017). Enhanced performance of the microalga *Chlorella sorokiniana* remotely induced by the plant growth-promoting bacteria *Azospirillum brasilense* and *Bacillus pumilus*. *Scientific Reports*, 7, 62-66. <https://doi.org/10.1038/srep41310>
- Amin, S. A., Hmelo, L. R., Van Tol, H. M., Durham, B. P., Carlson, L. T., Heal, K. R., Morales, R. L., Berthiaume, C. T., Parker, M. S., Djunaedi, B., Ingalls, A. E., Parsek, M. R., Moran, M. A., & Armbrust, E. V. (2015). Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature*, 522(7554), 62-66.

<https://doi.org/10.1038/nature14488>

- Anantharaman, K., Brown, C. T., Hug, L. A., Sharon, I., Castelle, C. J., Probst, A. J., Thomas, B. C., Singh, A., Wilkins, M. J., Karaoz, U., Brodie, E. L., Williams, K. H., Hubbard, S. S., & Banfield, J. F. (2016). Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. *Nature Communications*, 7. <https://doi.org/10.1038/ncomms13219>
- Andersen, R. A., Brett, R. W., Potter, D., & Sexton, J. P. (1998). Phylogeny of the Eustigmatophyceae based upon 18s rDNA, with emphasis on *Nannochloropsis*. *Protist*, 149(1), 265-276. [https://doi.org/10.1016/S1434-4610\(98\)70010-0](https://doi.org/10.1016/S1434-4610(98)70010-0)
- Anisimova, M., & Gascuel, O. (2006). Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Systematic Biology*, 55, 396-408. <https://doi.org/10.1080/10635150600755453>
- Arbib, Z., Ruiz, J., Alvarez, P., Garrido, C., Barragan, J., & Perales, J. A. (2012). *Chlorella stigmatophora* for urban wastewater nutrient removal and CO₂ abatement. *International Journal of Phytoremediation*, 14(7), 187-199. <https://doi.org/10.1080/15226514.2011.619237>
- Ashour, M., Elshobary, M. E., El-Shenody, R., Kamil, A. W., & Abomohra, A. E. F. (2019). Evaluation of a native oleaginous marine microalga *Nannochloropsis oceanica* for dual use in biodiesel production and aquaculture feed. *Biomass and Bioenergy*, 120, 176-185. <https://doi.org/10.1016/j.biombioe.2018.12.009>
- Aslam, A., Thomas-Hall, S. R., Mughal, T. A., & Schenk, P. M. (2017). Selection and adaptation of microalgae to growth in 100% unfiltered coal-fired flue gas. *Bioresource Technology*, 233, 271-283. <https://doi.org/10.1016/j.biortech.2017.02.111>
- Astafyeva, Y., Gurschke, M., Qi, M., Bergmann, L., Indenbirken, D., de Grahl, I., Katzowitsch, E., Reumann, S., Hanelt, D., Alawi, M., Streit, W. R., & Krohn, I. (2022). Microalgae and bacteria interaction—Evidence for division of diligence in the alga microbiota. *Microbiology Spectrum*, 10(4), e00633-22. <https://doi.org/10.1128/spectrum.00633-22>
- Ayswaria, R., Vijayan, J., & Nathan, V. K. (2023). Antimicrobial peptides derived from microalgae for combating antibiotic resistance: Current status and prospects. *Cell Biochemistry and Function*, 41, 142-151. <https://doi.org/10.1002/cbf.3779>
- Bağcı, C., Patz, S., & Huson, D. (2021). DIAMOND+MEGAN: Fast and easy taxonomic and functional analysis of short and long microbiome sequences. *Current Protocols*, 1(e59), 1–30. <https://doi.org/10.1002/cpz1.59>
- Batinovic, S., Rose, J. J. A., Ratcliffe, J., Seviour, R. J., & Petrovski, S. (2021). Cocultivation of an ultrasmall environmental parasitic bacterium with lytic ability against bacteria associated with wastewater foams. *Nature Microbiology*, 6(6), 703-711.

<https://doi.org/10.1038/s41564-021-00892-1>

- Barbosa, M. J., Janssen, M., Südfeld, C., D'Adamo, S., & Wijffels, R. H. (2023). Hypes, hopes, and the way forward for microalgal biotechnology. *Trends in Biotechnology*, 41(3), 452-471. <https://doi.org/10.1016/j.tibtech.2022.12.017>
- Bashan, Y., & Holguin, G. (1998). Proposal for the division of plant growth-promoting rhizobacteria into two classifications: Biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil Biology and Biochemistry*, 30(8–9), 975-1228. [https://doi.org/10.1016/S0038-0717\(97\)00187-9](https://doi.org/10.1016/S0038-0717(97)00187-9)
- Bell, W., & Mitchell, R. (1972). Chemotactic and growth responses of marine bacteria to algal extracellular products. *The Biological Bulletin*, 143(2), 265-277. <https://doi.org/10.2307/1540052>
- Bhattacharya, D., & Medlin, L. (1995). The phylogeny of plastids: A review based on comparisons of small-subunit ribosomal RNA coding regions. *Journal of Phycology*, 31(4), 489-498. <https://doi.org/10.1111/j.1529-8817.1995.tb02542.x>
- Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology and Biotechnology*, 28(4), 1327–1350. <https://doi.org/10.1007/s11274-011-0979-9>
- Biddanda, B., & Pomeroy, L. (1988). Microbial aggregation and degradation of phytoplankton-derived detritus in seawater. I. Microbial succession. *Marine Ecology Progress Series*, 42, 90-95. <https://doi.org/10.3354/meps042079>
- Bidle, K. D., & Fletcher, M. (1995). Comparison of free-living and particle-associated bacterial communities in the Chesapeake Bay by stable low-molecular-weight RNA analysis. *Applied and Environmental Microbiology*, 61(3), 944–952. <https://doi.org/10.1128/aem.61.3.944-952.1995>
- Billinton, N., & Knight, A. W. (2001). Seeing the wood through the trees: A review of techniques for distinguishing green fluorescent protein from endogenous autofluorescence. *Analytical Biochemistry*, 291(2), 175-197. <https://doi.org/10.1006/abio.2000.5006>
- Blanken, W., Janssen, M., Cuaresma, M., Libor, Z., Bhajji, T., & Wijffels, R. H. (2014). Biofilm growth of *Chlorella sorokiniana* in a rotating biological contactor based photobioreactor. *Biotechnology and Bioengineering*, 111(12), 2436-2445. <https://doi.org/10.1002/bit.25301>
- Bloembergen, G. V., & Lugtenberg, B. J. J. (2001). Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Current Opinion in Plant Biology*, 4(4), 343-350. [https://doi.org/10.1016/S1369-5266\(00\)00183-7](https://doi.org/10.1016/S1369-5266(00)00183-7)

- Bowers, R. M., Kyrpides, N. C., Stepanauskas, R., Harmon-Smith, M., Doud, D., Reddy, T. B. K., Schulz, F., Jarett, J., Rivers, A. R., Elie-Fadrosh, E. A., Tringe, S. G., Ivanova, N. N., Copeland, A., Clum, A., Becraft, E. D., Malmstrom, R. R., Birren, B., Podar, M., Bork, P., ... Woyke, T. (2017). Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. *Nature Biotechnology*, 35(8), 725–731. <https://doi.org/10.1038/nbt.3893>
- Bratbak, G., & Thingstad, T. F. (1985). Phytoplankton-bacteria interactions: an apparent paradox? Analysis of a model system with both competition and commensalism. *Marine Ecology Progress Series*, 25, 23–30. [https://doi.org/10.1016/0198-0254\(86\)91170-2](https://doi.org/10.1016/0198-0254(86)91170-2)
- Brennan, L., & Owende, P. (2010). Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, 14(2), 557-577. <https://doi.org/10.1016/j.rser.2009.10.009>
- Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., Olson, R., Overbeek, R., Parrello, B., Pusch, G. D., Shukla, M., Thomason, J. A., Stevens, R., Vonstein, V., Wattam, A. R., & Xia, F. (2015). RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Scientific Reports*, 5, 8365. <https://doi.org/10.1038/srep08365>
- Breuer, G., Lamers, P. P., Martens, D. E., Draaisma, R. B., & Wijffels, R. H. (2013). Effect of light intensity, pH, and temperature on triacylglycerol (TAG) accumulation induced by nitrogen starvation in *Scenedesmus obliquus*. *Bioresource Technology*, 143, 1-9. <https://doi.org/10.1016/j.biortech.2013.05.105>
- Brown, C. T., Hug, L. A., Thomas, B. C., Sharon, I., Castelle, C. J., Singh, A., Wilkins, M. J., Wrighton, K. C., Williams, K. H., & Banfield, J. F. (2015). Unusual biology across a group comprising more than 15% of domain Bacteria. *Nature*, 523(7559), 208–211. <https://doi.org/10.1038/nature14486>
- Bruckner, C. G., Rehm, C., Grossart, H. P., & Kroth, P. G. (2011). Growth and release of extracellular organic compounds by benthic diatoms depend on interactions with bacteria. *Environmental Microbiology*, 13(4), 1052-1063. <https://doi.org/10.1111/j.1462-2920.2010.02411.x>
- Buck, D., Weirather, J. L., de Cesare, M., Wang, Y., Piazza, P., Sebastiano, V., Wang, X. J., & Au, K. F. (2017). Comprehensive comparison of Pacific Biosciences and Oxford Nanopore Technologies and their applications to transcriptome analysis. *F1000Research*, 6, 100. <https://doi.org/10.12688/f1000research.10571.2>
- Bull, B. S., Schneiderman, M. A., & Brecher, G. (1965). Platelet counts with the Coulter counter. *American Journal of Clinical Pathology*, 44(6), 678–688. <https://doi.org/10.1093/ajcp/44.6.678>
- Cai, T., Ge, X., Park, S. Y., & Li, Y. (2013). Comparison of *Synechocystis* sp. PCC6803 and

- Nannochloropsis salina* for lipid production using artificial seawater and nutrients from anaerobic digestion effluent. *Bioresource Technology*, 144, 255-260.
<https://doi.org/10.1016/j.biortech.2013.06.101>
- Cao, Y., Wang, Y., Zheng, X., Li, F., & Bo, X. (2016). RevEcoR: An R package for the reverse ecology analysis of microbiomes. *BMC Bioinformatics*, 17(1), 1–6.
<https://doi.org/10.1186/s12859-016-1088-4>
- Cardon, Z. G., Peredo, E. L., Dohnalkova, A. C., Gershon, H. L., & Bezanilla, M. (2018). A model suite of green algae within the Scenedesmales for investigating contrasting desiccation tolerance and morphology. *Journal of Cell Science*, 131(7).
<https://doi.org/10.1242/jcs.212233>
- Carreira, C., Staal, M., Middelboe, M., & Brussaard, C. P. D. (2015). Autofluorescence imaging system to discriminate and quantify the distribution of benthic cyanobacteria and diatoms. *Limnology and Oceanography: Methods*, 13(4), 169-177.
<https://doi.org/10.1002/lom3.10016>
- Castelle, C. J., Brown, C. T., Anantharaman, K., Probst, A. J., Huang, R. H., & Banfield, J. F. (2018). Biosynthetic capacity, metabolic variety and unusual biology in the CPR and DPANN radiations. *Nature Reviews Microbiology*, 16(10), 169-177.
<https://doi.org/10.1038/s41579-018-0076-2>
- Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17(4), 540–552.
<https://doi.org/10.1093/oxfordjournals.molbev.a026334>
- Chae, S. R., Hwang, E. J., & Shin, H. S. (2006). Single cell protein production of *Euglena gracilis* and carbon dioxide fixation in an innovative photo-bioreactor. *Bioresource Technology*, 97(2), 322-329. <https://doi.org/10.1016/j.biortech.2005.02.037>
- Chang, T., Gavelis, G. S., Brown, J. M., & Stepanauskas, R. (2024). Genomic representativeness and chimerism in large collections of SAGs and MAGs of marine prokaryoplankton. *Microbiome*, 12(1), 1–14. <https://doi.org/10.1186/s40168-024-01848-3>
- Chang, Y. L., Nagarajan, D., Chen, J. H., Yen Chen, C., Wu, Y. J., Whang, L. M., Lee, D. J., & Chang, J. S. (2023). Microalgae-bacteria consortia for the treatment of raw dairy manure wastewater using a novel two-stage process: Process optimization and bacterial community analysis. *Chemical Engineering Journal*, 473, 145388.
<https://doi.org/10.1016/j.cej.2023.145388>
- Chaudhari, N. M., Overholt, W. A., Figueroa-Gonzalez, P. A., Taubert, M., Bornemann, T. L. V., Probst, A. J., Hölzer, M., Marz, M., & Küsel, K. (2021). The economical lifestyle of CPR bacteria in groundwater allows little preference for environmental drivers. *Environmental Microbiomes*, 16,24. <https://doi.org/10.1186/s40793-021-00395-w>

- Chaumeil, P. A., Mussig, A. J., Hugenholtz, P., & Parks, D. H. (2020). GTDB-Tk: A toolkit to classify genomes with the genome taxonomy database. *Bioinformatics*, 36(6), 1925–1927. <https://doi.org/10.1093/bioinformatics/btz848>
- Cheah, W. Y., Show, P. L., Chang, J. S., Ling, T. C., & Juan, J. C. (2015). Biosequestration of atmospheric CO₂ and flue gas-containing CO₂ by microalgae. *Bioresource Technology*, 184, 190-201. <https://doi.org/10.1016/j.biortech.2014.11.026>
- Cheng-Wu, Z., Zmora, O., Kopel, R., & Richmond, A. (2001). An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). *Aquaculture*, 195(1–2), 35-49. [https://doi.org/10.1016/S0044-8486\(00\)00533-0](https://doi.org/10.1016/S0044-8486(00)00533-0)
- Cheng, J., Xin, K., Wang, Z., Zhu, Y., Xia, R., Yang, W., & Liu, J. (2022). Improving biomass growth of *Nannochloropsis oceanica* with electrical treatment. *Journal of CO₂ Utilization*, 58, 101923. <https://doi.org/10.1016/j.jcou.2022.101923>
- Chevenet, F., Brun, C., Bañuls, A. L., Jacq, B., & Christen, R. (2006). TreeDyn: Towards dynamic graphics and annotations for analyses of trees. *BMC Bioinformatics*, 7, 439. <https://doi.org/10.1186/1471-2105-7-439>
- Chi, Y., & Takiguchi, Y. (2015). Comparison of the growth performance of *Nannochloropsis oceanica* IMET1 and *Nannochloropsis gaditana* CCMP526 under various culture conditions. *Journal of Plant Sciences*, 3(1), 9-13. <https://doi.org/10.11648/j.jps.20150301.12>
- Chiriac, M. C., Bulzu, P. A., Andrei, A. S., Okazaki, Y., Nakano, S., Haber, M., Kavagutti, V. S., Layoun, P., Ghai, R., & Salcher, M. M. (2022). Ecogenomics sheds light on diverse lifestyle strategies in freshwater CPR. *Microbiome*, 10, 84. <https://doi.org/10.1186/s40168-022-01274-3>
- Chivian, D., Jungbluth, S. P., Dehal, P. S., Wood-Charlson, E. M., Canon, R. S., Allen, B. H., Clark, M. M., Gu, T., Land, M. L., Price, G. A., Riehl, W. J., Sneddon, M. W., Sutormin, R., Zhang, Q., Cottingham, R. W., Henry, C. S., & Arkin, A. P. (2023). Metagenome-assembled genome extraction and analysis from microbiomes using KBase. *Nature Protocols*, 18(1), 208-238. <https://doi.org/10.1038/s41596-022-00747-x>
- Chklovski, A., Parks, D. H., Woodcroft, B. J., & Tyson, G. W. (2023). CheckM2: a rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. *Nature Methods*, 20(8), 1203-1212. <https://doi.org/10.1038/s41592-023-01940-w>
- Cho, D. H., Ramanan, R., Heo, J., Lee, J., Kim, B. H., Oh, H. M., & Kim, H. S. (2015). Enhancing microalgal biomass productivity by engineering a microalgal-bacterial community. *Bioresource Technology*, 175, 578-585. <https://doi.org/10.1016/j.biortech.2014.10.159>
- Cho, S. H., Lee, E., Ko, S. R., Jin, S., Song, Y., Ahn, C. Y., Oh, H. M., Cho, B. K., & Cho, S.

- (2020). Elucidation of the biosynthetic pathway of Vitamin B groups and potential secondary metabolite gene clusters via genome analysis of a marine bacterium *Pseudoruegeria* sp. M32A2M. *Journal of Microbiology and Biotechnology*, 30(4), 505-514. <https://doi.org/10.4014/JMB.1911.11006>
- Choi, D. H., Ahn, C., Jang, G. Il, Lapidus, A., Han, J., Reddy, T. B. K., Huntemann, M., Pati, A., Ivanova, N., Markowitz, V., Rohde, M., Tindall, B., Göker, M., Woyke, T., Klenk, H. P., Kyrpides, N. C., & Cho, B. C. (2015). High-quality draft genome sequence of *Gracilimonas tropica* CL-CB462T (DSM 19535T), isolated from a *Synechococcus* culture. *Standards in Genomic Sciences*, 10,98. <https://doi.org/10.1186/s40793-015-0088-8>
- Choi, Il H., Hwang, S. W., Kim, J., Park, B., Jin, E. S., Choi, I. G., & Sim, S. J. (2021). Augmented CO₂ tolerance by expressing a single H⁺-pump enables microalgal valorization of industrial flue gas. *Nature Communications*, 12,6049. <https://doi.org/10.1038/s41467-021-26325-5>
- Christenson, L. B., & Sims, R. C. (2012). Rotating algal biofilm reactor and spool harvester for wastewater treatment with biofuels by-products. *Biotechnology and Bioengineering*, 109(7), 1674-1684. <https://doi.org/10.1002/bit.24451>
- Chun, J., & Rainey, F. A. (2014). Integrating genomics into the taxonomy and systematics of the Bacteria and Archaea. *International Journal of Systematic and Evolutionary Microbiology*, 64, 316-324. <https://doi.org/10.1099/ijs.0.054171-0>
- Chuvochina, M., Rinke, C., Parks, D. H., Rappé, M. S., Tyson, G. W., Yilmaz, P., Whitman, W. B., & Hugenholtz, P. (2019). The importance of designating type material for uncultured taxa. *Systematic and Applied Microbiology*, 42(1), 15–21. <https://doi.org/10.1016/j.syapm.2018.07.003>
- Corteggiani Carpinelli, E., Telatin, A., Vitulo, N., Forcato, C., D'Angelo, M., Schiavon, R., Vezzi, A., Giacometti, G. M., Morosinotto, T., & Valle, G. (2014). Chromosome scale genome assembly and transcriptome profiling of *Nannochloropsis gaditana* in nitrogen depletion. *Molecular Plant*, 7(2), 323-335. <https://doi.org/10.1093/mp/sst120>
- Coyne, K. J., Wang, Y., & Johnson, G. (2022). Algicidal bacteria: A review of current knowledge and applications to control harmful algal blooms. *Frontiers in Microbiology*, 13, 871177. <https://doi.org/10.3389/fmicb.2022.871177>
- Croft, M. T., Lawrence, A. D., Raux-Deery, E., Warren, M. J., & Smith, A. G. (2005). Algae acquire vitamin B₁₂ through a symbiotic relationship with bacteria. *Nature*, 438(7064), 90-95. <https://doi.org/10.1038/nature04056>
- Croft, M. T., Warren, M. J., & Smith, A. G. (2006). Algae need their vitamins. *Eukaryotic Cell*, 5(8), 1175-1183. <https://doi.org/10.1128/EC.00097-06>
- Cuéllar-Franca, R. M., & Azapagic, A. (2015). Carbon capture, storage and utilisation

- technologies: A critical analysis and comparison of their life cycle environmental impacts. *Journal of CO₂ Utilization*, 9, 82-102. <https://doi.org/10.1016/j.jcou.2014.12.001>
- Daly, G., Decorosi, F., Viti, C., & Adessi, A. (2023). Shaping the phycosphere: Analysis of the EPS in diatom-bacterial co-cultures. *Journal of Phycology*, 59(4), 791-797. <https://doi.org/10.1111/jpy.13361>
- Damaraju, N., Miller, A. L., & Miller, D. E. (2024). Long-read DNA and RNA sequencing to streamline clinical genetic testing and reduce barriers to comprehensive genetic testing. *Journal of Applied Laboratory Medicine*, 9(1), 138–150. <https://doi.org/10.1093/jalm/jfad107>
- Danczak, R. E., Johnston, M. D., Kenah, C., Slattery, M., Wrighton, K. C., & Wilkins, M. J. (2017). Members of the Candidate Phyla Radiation are functionally differentiated by carbon- and nitrogen-cycling capabilities. *Microbiome*, 5, 112. <https://doi.org/10.1186/s40168-017-0331-1>
- Daneshvar, E., Wicker, R. J., Show, P. L., & Bhatnagar, A. (2022). Biologically-mediated carbon capture and utilization by microalgae towards sustainable CO₂ biofixation and biomass valorization – A review. *Chemical Engineering Journal*, 427, 130884. <https://doi.org/10.1016/j.cej.2021.130884>
- Dao, G. H., Wu, G. X., Wang, X. X., Zhang, T. Y., Zhan, X. M., & Hu, H. Y. (2018). Enhanced microalgae growth through stimulated secretion of indole acetic acid by symbiotic bacteria. *Algal Research*, 33, 345-351. <https://doi.org/10.1016/j.algal.2018.06.006>
- Dashkova, V., Segev, E., Malashenkov, D., Kolter, R., Vorobjev, I., & Barteneva, N. S. (2016). Microalgal cytometric analysis in the presence of endogenous autofluorescent pigments. *Algal Research*, 19, 370-380. <https://doi.org/10.1016/j.algal.2016.05.013>
- De-Bashan, L. E., Antoun, H., & Bashan, Y. (2008). Involvement of indole-3-acetic acid produced by the growth-promoting bacterium *Azospirillum* spp. in promoting growth of *Chlorella vulgaris*. *Journal of Phycology*, 44(4), 938-947. <https://doi.org/10.1111/j.1529-8817.2008.00533.x>
- De Coster, W., & Rademakers, R. (2023). NanoPack2: population-scale evaluation of long-read sequencing data. *Bioinformatics*, 39(5), btad311. <https://doi.org/10.1093/bioinformatics/btad311>
- De Lajudie, P., Laurent-Fulele, E., Willems, A., Torck, U., Coopman, R., Collins, M. D., Kersters, K., Dreyfus, B., & Gillis, M. (1998). *Allorhizobium undicola* gen. nov., sp. nov., nitrogen-fixing bacteria that efficiently nodulate *Neptunia natans* in Senegal. *International Journal of Systematic Bacteriology*, 48(4), 1277-1290. <https://doi.org/10.1099/00207713-48-4-1277>
- de Morais, M. G., & Costa, J. A. V. (2007a). Biofixation of carbon dioxide by *Spirulina* sp. and

- Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *Journal of Biotechnology*, 129(3), 439-445. <https://doi.org/10.1016/j.jbiotec.2007.01.009>
- de Morais, M. G., & Costa, J. A. V. (2007b). Carbon dioxide fixation by *Chlorella kessleri*, *C. vulgaris*, *Scenedesmus obliquus* and *Spirulina* sp. cultivated in flasks and vertical tubular photobioreactors. *Biotechnology Letters*, 29(9), 1349–1352. <https://doi.org/10.1007/s10529-007-9394-6>
- de Morais, M. G., & Costa, J. A. V. (2007c). Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. *Energy Conversion and Management*, 48(7), 2169-2173. <https://doi.org/10.1016/j.enconman.2006.12.011>
- de Muinck, E. J., Trosvik, P., Gilfillan, G. D., Hov, J. R., & Sundaram, A. Y. M. (2017). A novel ultra high-throughput 16S rRNA gene amplicon sequencing library preparation method for the Illumina HiSeq platform. *Microbiome*, 5, 68. <https://doi.org/10.1186/s40168-017-0279-1>
- DeLong, E. F., Franks, D. G., & Alldredge, A. L. (1993). Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnology and Oceanography*, 38(5), 924-934. <https://doi.org/10.4319/lo.1993.38.5.0924>
- Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., & Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, 36(2), W465–W469. <https://doi.org/10.1093/nar/gkn180>
- Dereeper, Alexis, Audic, S., Claverie, J. M., & Blanc, G. (2010). BLAST-EXPLORER helps you building datasets for phylogenetic analysis. *BMC Evolutionary Biology*, 10, 8. <https://doi.org/10.1186/1471-2148-10-8>
- do Carmo Cesário, C., Soares, J., Cossolin, J. F. S., Almeida, A. V. M., Bermudez Sierra, J. J., de Oliveira Leite, M., Nunes, M. C., Serrão, J. E., Martins, M. A., & dos Reis Coimbra, J. S. (2022). Biochemical and morphological characterization of freshwater microalga *Tetrademus obliquus* (Chlorophyta: Chlorophyceae). *Protoplasma*, 259(4), 937–948. <https://doi.org/10.1007/s00709-021-01712-3>
- Do Nascimento, M., Dublan, M. de los A., Ortiz-Marquez, J. C. F., & Curatti, L. (2013). High lipid productivity of an *Ankistrodesmus-Rhizobium* artificial consortium. *Bioresource Technology*, 146, 400-407. <https://doi.org/10.1016/j.biortech.2013.07.085>
- Doi, H., Takahara, T., Minamoto, T., Matsushashi, S., Uchii, K., & Yamanaka, H. (2015). Droplet digital polymerase chain reaction (PCR) outperforms real-time PCR in the detection of environmental DNA from an invasive fish species. *Environmental Science and Technology*, 49(9), 5601-5608. <https://doi.org/10.1021/acs.est.5b00253>
- Dong, H. P., Williams, E., Wang, D. Z., Xie, Z. X., Hsia, R. C., Jenck, A., Halden, R., Li, J.,

- Chen, F., & Place, A. R. (2013). Responses of *Nannochloropsis oceanica* IMET1 to long-term nitrogen starvation and recovery. *Plant Physiology*, 162(2), 1110–1126. <https://doi.org/10.1104/pp.113.214320>
- Doucha, J., Straka, F., & Lívanský, K. (2005). Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin-layer photobioreactor. *Journal of Applied Phycology*, 17(5), 403–412. <https://doi.org/10.1007/s10811-005-8701-7>
- Dow, L. (2021). How do quorum-sensing signals mediate algae–bacteria interactions? *Microorganisms*, 9(7), 1391. <https://doi.org/10.3390/microorganisms9071391>
- Driscoll, T. P., Verhoeve, V. I., Guillotte, M. L., Lehman, S. S., Rennoll, S. A., Beier-Sexton, M., Rahman, M. S., Azad, A. F., & Gillespie, J. J. (2017). Wholly rickettsial! Reconstructed metabolic profile of the quintessential bacterial parasite of eukaryotic cells. *mBio*, 8(5), e00859-17. <https://doi.org/10.1128/mBio.00859-17>
- Duarte, J. H., de Moraes, E. G., Radmann, E. M., & Costa, J. A. V. (2017). Biological CO₂ mitigation from coal power plant by *Chlorella fusca* and *Spirulina* sp. *Bioresource Technology*, 234, 472–475. <https://doi.org/10.1016/j.biortech.2017.03.066>
- Duarte, J. H., Fanka, L. S., & Costa, J. A. V. (2020). CO₂ biofixation via *Spirulina* sp. cultures: evaluation of initial biomass concentration in tubular and raceway photobioreactors. *Bioenergy Research*, 13(3), 939–943. <https://doi.org/10.1007/s12155-020-10117-8>
- Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32(5), 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Elwood, H. J., Olsen, G. J., & Sogin, M. L. (1985). The small-subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytricha nova* and *Stylonychia pustulata*. *Molecular Biology and Evolution*, 2(5), 399–410. <https://doi.org/10.1093/oxfordjournals.molbev.a040362>
- Esteves, A. F., Pires, J. C. M., & Gonçalves, A. L. (2021). Current utilization of microalgae in the food industry beyond direct human consumption. *Current and Potential Applications*, 199–248. <https://doi.org/10.1016/B978-0-12-821080-2.00005-8>
- Even, C., Hadroug, D., Boumlaik, Y., & Simon, G. (2022). Microalgae-based Bioenergy with Carbon Capture and Storage quantified as a Negative Emissions Technology. *Energy Nexus*, 7, 100117. <https://doi.org/10.1016/j.nexus.2022.100117>
- Fandino, L. B., Riemann, L., Steward, G. F., Long, R. A., & Azam, F. (2001). Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing. *Aquatic Microbial Ecology*, 23(2), 119–130. <https://doi.org/10.3354/ame023119>
- Fawley, K. P., & Fawley, M. W. (2007). Observations on the diversity and ecology of freshwater

- Nannochloropsis* (Eustigmatophyceae), with descriptions of new taxa. *Protist*, 158(3), 325-336. <https://doi.org/10.1016/j.protis.2007.03.003>
- Feng, X., & Li, H. (2024). Evaluating and improving the representation of bacterial contents in long-read metagenome assemblies. *Genome Biology*, 25(1), 1–18. <https://doi.org/10.1186/s13059-024-03234-6>
- Fouilland, E., Tolosa, I., Bonnet, D., Bouvier, C., Bouvier, T., Bouvy, M., Got, P., Le Floch, E., Mostajir, B., Roques, C., Sempéré, R., Sime-Ngando, T., & Vidussi, F. (2014). Bacterial carbon dependence on freshly produced phytoplankton exudates under different nutrient availability and grazing pressure conditions in coastal marine waters. *FEMS Microbiology Ecology*, 87(3), 757–769. <https://doi.org/10.1111/1574-6941.12262>
- Fuhrman, J. A., & Azam, F. (1982). Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluation and field results. *Marine Biology*, 66(2), 109–120. <https://doi.org/10.1007/BF00397184>
- Fuqua, C., Parsek, M. R., & Greenberg, E. P. (2001). Regulation of gene expression by cell-to-cell communication: Acyl-homoserine lactone quorum sensing. *Annual Review of Genetics*, 35, 439-468. <https://doi.org/10.1146/annurev.genet.35.102401.090913>
- Gan, X., Shen, G., Xin, B., & Li, M. (2016). Simultaneous biological desalination and lipid production by *Scenedesmus obliquus* cultured with brackish water. *Desalination*, 400, 1-6. <https://doi.org/10.1016/j.desal.2016.09.012>
- Ge, Y., Liu, J., & Tian, G. (2011). Growth characteristics of *Botryococcus braunii* 765 under high CO₂ concentration in photobioreactor. *Bioresource Technology*, 102(1), 130-134. <https://doi.org/10.1016/j.biortech.2010.06.051>
- Geng, Y., Xiong, Z., Yang, L., Lian, C. A., Pavlostathis, S. G., Qiu, Z., Chen, H., Luo, Q., Liu, Y., Liu, Z., Shao, P., Zou, J. P., Jiang, H., Luo, S., Yu, K., & Luo, X. (2024). Bidirectional enhancement of nitrogen removal by indigenous synergetic microalgal-bacterial consortia in harsh low-C/N wastewater. *Environmental Science and Technology*, 58(12). <https://doi.org/10.1021/acs.est.3c10322>
- Gios, E., Mosley, O. E., Weaver, L., Close, M., Daughney, C., & Handley, K. M. (2023). Ultra-small bacteria and archaea exhibit genetic flexibility towards groundwater oxygen content, and adaptations for attached or planktonic lifestyles. *ISME Communications*, 3(1). <https://doi.org/10.1038/s43705-023-00223-x>
- Glaeser, S. P., & Kämpfer, P. (2015). Multilocus sequence analysis (MLSA) in prokaryotic taxonomy. *Systematic and Applied Microbiology*, 38(4), 237-245. <https://doi.org/10.1016/j.syapm.2015.03.007>
- Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica*,

2012. <https://doi.org/10.6064/2012/963401>
- Gómez-Serrano, C., Morales-Amaral, M. M., Acién, F. G., Escudero, R., Fernández-Sevilla, J. M., & Molina-Grima, E. (2015). Utilization of secondary-treated wastewater for the production of freshwater microalgae. *Applied Microbiology and Biotechnology*, 99(16). <https://doi.org/10.6064/2012/963401>
- Gonzalez, L. E., & Bashan, Y. (2000). Increased growth of the microalga *Chlorella vulgaris* when coimmobilized and cocultured in alginate beads with the plant-growth-promoting bacterium *Azospirillum brasilense*. *Applied and Environmental Microbiology*, 66(4). <https://doi.org/10.1128/AEM.66.4.1527-1531.2000>
- Goodwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews Genetics*, 17(6), 333–351. <https://doi.org/10.1038/nrg.2016.49>
- Gould, S. B., Waller, R. F., & McFadden, G. I. (2008). Plastid evolution. *Annual Review of Plant Biology*, 59, 491–517. <https://doi.org/10.1146/annurev.arplant.59.032607.092915>
- Grant, M. A. A., Kazamia, E., Cicuta, P., & Smith, A. G. (2014). Direct exchange of vitamin B₁₂ is demonstrated by modelling the growth dynamics of algal-bacterial cocultures. *ISME Journal*, 8(7), 1418–1427. <https://doi.org/10.1038/ismej.2014.9>
- Gris, B., Morosinotto, T., Giacometti, G. M., Bertucco, A., & Sforza, E. (2014). Cultivation of *Scenedesmus obliquus* in photobioreactors: Effects of light intensities and light-dark cycles on growth, productivity, and biochemical composition. *Applied Biochemistry and Biotechnology*, 172(5), 2377–2389. <https://doi.org/10.1007/s12010-013-0679-z>
- Grossart, H. P., Levold, F., Allgaier, M., Simon, M., & Brinkhoff, T. (2005). Marine diatom species harbour distinct bacterial communities. *Environmental Microbiology*, 7(6), 860–873. <https://doi.org/10.1111/j.1462-2920.2005.00759.x>
- Guedes, A. C., Amaro, H. M., Pereira, R. D., & Malcata, F. X. (2011). Effects of temperature and pH on growth and antioxidant content of the microalga *Scenedesmus obliquus*. *Biotechnology Progress*, 27(5), 1218–1224. <https://doi.org/10.1002/btpr.649>
- Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, 52(5): 696–704. <https://doi.org/10.1080/10635150390235520>
- Han, B. P., Virtanen, M., Koponen, J., & Straškraba, M. (2000). Effect of photoinhibition on algal photosynthesis: A dynamic model. *Journal of Plankton Research*, 22(5), 865–885. <https://doi.org/10.1093/plankt/22.5.865>
- Han, J. R., Zhao, J. X., Wang, Z. J., Chen, G. J., & Du, Z. J. (2017). *Algoriphagus resistens* sp.

- nov., isolated from marine sediment. *International Journal of Systematic and Evolutionary Microbiology*, 67(5), 1275–1280. <https://doi.org/10.1099/ijsem.0.001805>
- Han, X., Rusconi, N., Ali, P., Pagkatipunan, K., & Chen, F. (2017). Nutrients extracted from chicken manure accelerate growth of *Scenedesmus obliquus* HTB1. *Green and Sustainable Chemistry*, 7(02), 101-113. <https://doi.org/10.4236/gsc.2017.72009>
- Handelsman, J. (2004). Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews*, 68(4), 669-685. <https://doi.org/10.1128/membr.68.4.669-685.2004>
- Haro-Moreno, J. M., Cabello-Yeves, P. J., Garcillán-Barcia, M. P., Zakharenko, A., Zemskaya, T. I., & Rodriguez-Valera, F. (2023). A novel and diverse group of *Candidatus* Patescibacteria from bathypelagic Lake Baikal revealed through long-read metagenomics. *Environmental Microbiome*, 18(1), 1–12. <https://doi.org/10.1186/s40793-023-00473-1>
- He, X., McLean, J. S., Edlund, A., Yooseph, S., Hall, A. P., Liu, S. Y., Dorrestein, P. C., Esquenazi, E., Hunter, R. C., Cheng, G., Nelson, K. E., Lux, R., & Shi, W. (2015). Cultivation of a human-associated TM7 phylotype reveals a reduced genome and epibiotic parasitic lifestyle. *Proceedings of the National Academy of Sciences of the United States of America*, 112(1). <https://doi.org/10.1073/pnas.1419038112>
- He, C., Keren, R., Whittaker, M. L., Farag, I. F., Doudna, J. A., Cate, J. H. D., & Banfield, J. F. (2021). Genome-resolved metagenomics reveals site-specific diversity of episymbiotic CPR bacteria and DPANN archaea in groundwater ecosystems. *Nature Microbiology*, 6(3), 354–365. <https://doi.org/10.1038/s41564-020-00840-5>
- He, S., Crans, V. L., & Jonikas, M. C. (2023). The pyrenoid: The eukaryotic CO₂-concentrating organelle. *Plant Cell*, 35(9), 3236–3259. <https://doi.org/10.1093/plcell/koad157>
- He, X., McLean, J. S., Edlund, A., Yooseph, S., Hall, A. P., Liu, S. Y., Dorrestein, P. C., Esquenazi, E., Hunter, R. C., Cheng, G., Nelson, K. E., Lux, R., & Shi, W. (2015). Cultivation of a human-associated TM7 phylotype reveals a reduced genome and epibiotic parasitic lifestyle. *Proceedings of the National Academy of Sciences of the United States of America*, 112(1), 244-249. <https://doi.org/10.1073/pnas.1419038112>
- Hedlund, B. P., Chuvochina, M., Hugenholtz, P., Konstantinidis, K. T., Murray, A. E., Palmer, M., Parks, D. H., Probst, A. J., Reysenbach, A. L., Rodriguez-R, L. M., Rossello-Mora, R., Sutcliffe, I. C., Venter, S. N., & Whitman, W. B. (2022). SeqCode: a nomenclatural code for prokaryotes described from sequence data. *Nature Microbiology*, 7(10), 1702–1708. <https://doi.org/10.1038/s41564-022-01214-9>
- Hernandez, J. P., de-Bashan, L. E., Rodriguez, D. J., Rodriguez, Y., & Bashan, Y. (2009). Growth promotion of the freshwater microalga *Chlorella vulgaris* by the nitrogen-fixing, plant growth-promoting bacterium *Bacillus pumilus* from arid zone soils. *European Journal of Soil Biology*, 45(1), 88-93. <https://doi.org/10.1016/j.ejsobi.2008.08.004>

- Hindersin, S., Leupold, M., Kerner, M., & Hanelt, D. (2013). Irradiance optimization of outdoor microalgal cultures using solar tracked photobioreactors. *Bioprocess and Biosystems Engineering*, 36(3), 345–355. <https://doi.org/10.1007/s00449-012-0790-5>
- Hindersin, S., Leupold, M., Kerner, M., & Hanelt, D. (2014). Key parameters for outdoor biomass production of *Scenedesmus obliquus* in solar tracked photobioreactors. *Journal of Applied Phycology*, 26(6), 2315–2325. <https://doi.org/10.1007/s10811-014-0261-2>
- Hindson, B. J., Ness, K. D., Masquelier, D. A., Belgrader, P., Heredia, N. J., Makarewicz, A. J., Bright, I. J., Lucero, M. Y., Hiddessen, A. L., Legler, T. C., Kitano, T. K., Hodel, M. R., Petersen, J. F., Wyatt, P. W., Steenblock, E. R., Shah, P. H., Bousse, L. J., Troup, C. B., Mellen, J. C., ... Colston, B. W. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Analytical Chemistry*, 83(22), 8604–8610. <https://doi.org/10.1021/ac202028g>
- Ho, S. H., Chen, W. M., & Chang, J. S. (2010). *Scenedesmus obliquus* CNW-N as a potential candidate for CO₂ mitigation and biodiesel production. *Bioresource Technology*, 101(22), 8725–8730. <https://doi.org/10.1016/j.biortech.2010.06.112>
- Ho, S. H., Chen, C. Y., Lee, D. J., & Chang, J. S. (2011). Perspectives on microalgal CO₂-emission mitigation systems - A review. *Biotechnology Advances*, 29(2), 189–198. <https://doi.org/10.1016/j.biotechadv.2010.11.001>
- Ho, S. H., Chen, C. Y., & Chang, J. S. (2012). Effect of light intensity and nitrogen starvation on CO₂ fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. *Bioresource Technology*, 113, 244–252. <https://doi.org/10.1016/j.biortech.2011.11.133>
- Ho, S. H., Chang, J. S., Lai, Y. Y., & Chen, C. N. N. (2014). Achieving high lipid productivity of a thermotolerant microalga *Desmodesmus* sp. F2 by optimizing environmental factors and nutrient conditions. *Bioresource Technology*, 156, 108–116. <https://doi.org/10.1016/j.biortech.2014.01.017>
- Ho, S. H., Chen, Y. Di, Qu, W. Y., Liu, F. Y., & Wang, Y. (2019). Algal culture and biofuel production using wastewater. *Biomass, Biofuels, Biochemicals: Biofuels from Algae*, 167–198. <https://doi.org/10.1016/B978-0-444-64192-2.00008-1>
- Hoang, A. T., Sirohi, R., Pandey, A., Nižetić, S., Lam, S. S., Chen, W. H., Luque, R., Thomas, S., Arıcı, M., & Pham, V. V. (2022). Biofuel production from microalgae: Challenges and chances. *Phytochemistry Reviews*, 22, 1089–1126. <https://doi.org/10.1007/s11101-022-09819-y>
- Hodaifa, G., Martínez, M. E., & Sánchez, S. (2008). Use of industrial wastewater from olive-oil extraction for biomass production of *Scenedesmus obliquus*. *Bioresource Technology*, 99(5), 1111–1117. <https://doi.org/10.1016/j.biortech.2007.02.020>

- Hodaifa, G., Martínez, M. E., & Sánchez, S. (2010). Influence of temperature on growth of *Scenedesmus obliquus* in diluted olive mill wastewater as culture medium. *Engineering in Life Sciences*, 10(3), 257-264. <https://doi.org/10.1002/elsc.201000005>
- Hu, H., Kristensen, J. M., Herbold, C. W., Pjevac, P., Kitzinger, K., Hausmann, B., Dueholm, M. K. D., Nielsen, P. H., & Wagner, M. (2024). Global abundance patterns, diversity, and ecology of Patescibacteria in wastewater treatment plants. *Microbiome*, 12(1), 1–16. <https://doi.org/10.1186/s40168-024-01769-1>
- Hu, J., Wang, D., Li, J., Jing, G., Ning, K., & Xu, J. (2014). Genome-wide identification of transcription factors and transcription-factor binding sites in oleaginous microalgae *Nannochloropsis*. *Scientific Reports*, 4. <https://doi.org/10.1038/srep05454>
- Huang, Y., Chen, R., Zhang, Z., Qi, Y., Guan, Y., Ma, X., Wei, Q., & Yang, G. (2024). Enhancing wastewater treatment with a novel separated algae membrane oxygenated activated sludge reactor. *Journal of Water Process Engineering*, 61(100), 105283. <https://doi.org/10.1016/j.jwpe.2024.105283>
- Hug, L. A., Baker, B. J., Anantharaman, K., Brown, C. T., Probst, A. J., Castelle, C. J., Butterfield, C. N., Hernsdorf, A. W., Amano, Y., Ise, K., Suzuki, Y., Dudek, N., Relman, D. A., Finstad, K. M., Amundson, R., Thomas, B. C., & Banfield, J. F. (2016). A new view of the tree of life. *Nature Microbiology*, 1(5). <https://doi.org/10.1038/nmicrobiol.2016.48>
- Hughes, R. A., Jin, X., Zhang, Y., Zhang, R., Tran, S., Williams, P. G., Lindsey, J. S., & Miller, E. S. (2018). Genome sequence, metabolic properties and cyanobacterial attachment of *Porphyrobacter* sp. Ht-58-2 isolated from a filamentous cyanobacterium–microbial consortium. *Microbiology*, 164(10). <https://doi.org/10.1099/mic.0.000706>
- Hyatt, D., Chen, G. L., LoCascio, P. F., Land, M. L., Larimer, F. W., & Hauser, L. J. (2010). Prodigal: Prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics*, 11. <https://doi.org/10.1186/1471-2105-11-119>
- Hyka, P., Lickova, S., Přibyl, P., Melzoch, K., & Kovar, K. (2013). Flow cytometry for the development of biotechnological processes with microalgae. *Biotechnology Advances*, 13(1), 2-16. <https://doi.org/10.1016/j.biotechadv.2012.04.007>
- Iglina, T., Iglina, P., & Pashchenko, D. (2022). Industrial CO₂ capture by algae: A review and recent advances. *Sustainability*, 14(7), 3801. <https://doi.org/10.3390/su14073801>
- Jacob, M., Thomas, P. K., Giebel, H.-A., Billerbeck, S., Simon, M., Striebel, M., & Dlugosch, L. (2024). Cross-domain diversity effects: linking diatom species richness, intraspecific richness, and biomass production to host-associated bacterial diversity. *ISME Communications*, 4(1), 1–12. <https://doi.org/10.1093/ismeco/ycae046>
- Jang, J., Ashida, N., Kai, A., Isobe, K., Nishizawa, T., Otsuka, S., Yokota, A., Senoo, K., & Ishii,

- S. (2018). Presence of cu-type (Nirk) and cd1-type (nirs) nitrite reductase genes in the denitrifying bacterium *Bradyrhizobium nitroreducens* sp. nov. *Microbes and Environments*, 33(3), 326-331. <https://doi.org/10.1264/jsme2.ME18039>
- Jasti, S., Sieracki, M. E., Poulton, N. J., Giewat, M. W., & Rooney-Varga, J. N. (2005). Phylogenetic diversity and specificity of bacteria closely associated with *Alexandrium* spp. and other phytoplankton. *Applied and Environmental Microbiology*, 71(7), 3483-3494. <https://doi.org/10.1128/AEM.71.7.3483-3494.2005>
- Jeon, M. S., Han, S. Il, Ahn, J. W., Jung, J. H., Choi, J. S., & Choi, Y. E. (2023). Endophyte *Bacillus tequilensis* improves the growth of microalgae *Haematococcus lacustris* by regulating host cell metabolism. *Bioresource Technology*, 387. <https://doi.org/10.1016/j.biortech.2023.129546>
- Ji, B., & Liu, C. (2022). CO₂ improves the microalgal-bacterial granular sludge towards carbon-negative wastewater treatment. *Water Research*, 208. <https://doi.org/10.1016/j.watres.2021.117865>
- Jia, J., Han, D., Gerken, H. G., Li, Y., Sommerfeld, M., Hu, Q., & Xu, J. (2015). Molecular mechanisms for photosynthetic carbon partitioning into storage neutral lipids in *Nannochloropsis oceanica* under nitrogen-depletion conditions. *Algal Research*, 7, 66-77. <https://doi.org/10.1016/j.algal.2014.11.005>
- Jiang, L., Luo, S., Fan, X., Yang, Z., & Guo, R. (2011). Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO₂. *Applied Energy*, 88(10), 3336-3341. <https://doi.org/10.1016/j.apenergy.2011.03.043>
- Jiang, Y., Zhang, W., Wang, J., Chen, Y., Shen, S., & Liu, T. (2013). Utilization of simulated flue gas for cultivation of *Scenedesmus dimorphus*. *Bioresource Technology*, 128, 359-364. <https://doi.org/10.1016/j.biortech.2012.10.119>
- Jonas, L., Lee, Y., Mroz, R., Hill, R. T., Li, Y., Jonas, L., Lee, Y., Mroz, R., Hill, R. T., & Li, Y. (2025a). *Nannochloropsis oceanica* IMET1 and its bacterial symbionts for carbon capture, utilization, and storage: biomass and calcium carbonate production under high pH and high alkalinity. *Applied and Environmental Microbiology* [In Press].
- Jonas, L., Lee, Y.-Y., Bachvaroff, T., Hill, R. T., & Li, Y. (2025b). Two novel Patescibacteria: *Phycocordibacter aenigmaticus* gen. nov. sp. nov. and *Minuscolum obligatum* gen. nov. sp. nov., both associated with microalgae optimized for carbon dioxide sequestration from flue gas. *mBio*. [In Review].
- Jørgensen, T. S., Mohite, O. S., Sterndorff, E. B., Alvarez-Arevalo, M., Blin, K., Booth, T. J., Charusanti, P., Faurdal, D., Hansen, T. Ø., Nuhamunada, M., Mourched, A.-S., Palsson, B. Ø., & Weber, T. (2024). A treasure trove of 1034 actinomycete genomes. *Nucleic Acids Research*, 52, 7487–7503. <https://doi.org/10.1093/nar/gkae523>

- Kaewkannetra, P., Enmak, P., & Chiu, T. (2012). The effect of CO₂ and salinity on the cultivation of *Scenedesmus obliquus* for biodiesel production. *Biotechnology and Bioprocess Engineering*, 17(3), 591–597. <https://doi.org/10.1007/s12257-011-0533-5>
- Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*, 28(1), 27–30. <https://doi.org/10.1093/nar/28.1.27>
- Kawafune, K., Hongoh, Y., & Nozaki, H. (2014). A rickettsial endosymbiont inhabiting the cytoplasm of *Volvox carteri* (Volvocales, Chlorophyceae). *Phycologia*, 53(1). <https://doi.org/10.2216/13-193.1>
- Kazamia, E., Czesnick, H., Nguyen, T. T. Van, Croft, M. T., Sherwood, E., Sasso, S., Hodson, S. J., Warren, M. J., & Smith, A. G. (2012). Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation. *Environmental Microbiology*, 14(6), 1466–1476. <https://doi.org/10.1111/j.1462-2920.2012.02733.x>
- Kim, B. H., Ramanan, R., Cho, D. H., Oh, H. M., & Kim, H. S. (2014). Role of *Rhizobium*, a plant growth promoting bacterium, in enhancing algal biomass through mutualistic interaction. *Biomass and Bioenergy*, 69, 95–105. <https://doi.org/10.1016/j.biombioe.2014.07.015>
- Kim, J., Yoon, S. H., Yang, K. H., Kim, S., & Lee, S. S. (2020). *Algoriphagus aquimaris* sp. nov., isolated from seashore sand. *International Journal of Systematic and Evolutionary Microbiology*, 70(2), 721–731. <https://doi.org/10.1099/ijsem.0.003793>
- Kim, K. H., Kim, J. M., Baek, J. H., Jeong, S. E., Kim, H., Yoon, H. S., & Jeon, C. O. (2024). Metabolic relationships between marine red algae and algae-associated bacteria. *Marine Life Science and Technology*, 6(2), 298–314. <https://doi.org/10.1007/s42995-024-00227-z>
- Kishimoto, N., Kosako, Y., & Tano, T. (1991). *Acidobacterium capsulatum* gen. nov., sp. nov.: An acidophilic chemoorganotrophic bacterium containing menaquinone from acidic mineral environment. *Current Microbiology*, 22(1), 1–7. <https://doi.org/10.1007/BF02106205>
- Kloepper, J. W. (1980). Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. *Phytopathology*, 70(11). <https://doi.org/10.1094/phyto-70-1078>
- Kogure, K., Simidu, U., & Taga, N. (1981). Bacterial attachment to phytoplankton in sea water. *Journal of Experimental Marine Biology and Ecology*, 56(2–3), 197–204. [https://doi.org/10.1016/0022-0981\(81\)90189-1](https://doi.org/10.1016/0022-0981(81)90189-1)
- Kolmogorov, M., Yuan, J., Lin, Y., & Pevzner, P. A. (2019). Assembly of long, error-prone reads using repeat graphs. *Nature Biotechnology*, 37(5), 540–546. <https://doi.org/10.1038/s41587-019-0072-8>
- Kong, W., Kong, J., Lyu, H., Ma, J., Yuan, P., Wang, Z., Shen, B., & Feng, S. (2021).

- Integrating municipal wastewater treatment with CO₂ fixation and fatty acid production by cultivating *Tetradismus obliquus*. *Journal of Cleaner Production*, 320.
<https://doi.org/10.1016/j.jclepro.2021.128916>
- Konstantinidis, K. T., Rosselló-Móra, R., & Amann, R. (2017). Uncultivated microbes in need of their own taxonomy. *ISME Journal*, 11(11), 2399–2406.
<https://doi.org/10.1038/ismej.2017.113>
- Krohn-Molt, I., Alawi, M., Förstner, K. U., Wiegandt, A., Burkhardt, L., Indenbirken, D., Thieß, M., Grundhoff, A., Kehr, J., Tholey, A., & Streit, W. R. (2017). Insights into microalga and bacteria interactions of selected phycosphere biofilms using metagenomic, transcriptomic, and proteomic approaches. *Frontiers in Microbiology*, 8.
<https://doi.org/10.3389/fmicb.2017.01941>
- Krohn-Molt, I., Wemheuer, B., Alawi, M., Poehlein, A., Güllert, S., Schmeisser, C., Pommerening-Röser, A., Grundhoff, A., Daniel, R., Hanelt, D., & Streit, W. R. (2013). Metagenome survey of a multispecies and alga-associated biofilm revealed key elements of bacterial-algal interactions in photobioreactors. *Applied and Environmental Microbiology*, 79(20), 6196-6206. <https://doi.org/10.1128/AEM.01641-13>
- Kuckshinrichs, W. (2015). Carbon capture and utilization as an option for climate change mitigation: Integrated technology assessment. *Carbon Capture, Storage and Use: Technical, Economic, Environmental and Societal Perspectives*, 1-9.
https://doi.org/10.1007/978-3-319-11943-4_1
- Kumar, A., Yuan, X., Sahu, A. K., Ergas, S. J., Van Langenhove, H., & Dewulf, J. (2010a). A hollow fiber membrane photo-bioreactor for CO₂ sequestration from combustion gas coupled with wastewater treatment: A process engineering approach. *Journal of Chemical Technology and Biotechnology*, 85(3), 387-394. <https://doi.org/10.1002/jctb.2332>
- Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., Malcata, F. X., & van Langenhove, H. (2010b). Enhanced CO₂ fixation and biofuel production via microalgae: Recent developments and future directions. *Trends in Biotechnology*, 28(7), 371–380.
<https://doi.org/10.1016/j.tibtech.2010.04.004>
- Kumar, K., Banerjee, D., & Das, D. (2014). Carbon dioxide sequestration from industrial flue gas by *Chlorella sorokiniana*. *Bioresource Technology*, 152, 225-233.
<https://doi.org/10.1016/j.biortech.2013.10.098>
- Kumar, N., & Shukla, P. (2024). Microalgal multiomics-based approaches in bioremediation of hazardous contaminants. *Environmental Research*, 247.
<https://doi.org/10.1016/j.envres.2024.118135>
- Kuroda, K., Yamamoto, K., Nakai, R., Hirakata, Y., Kubota, K., Nobu, M. K., & Narihiro, T. (2022). Symbiosis between *Candidatus* Patescibacteria and archaea discovered in wastewater-treating bioreactors. *mBio*, 13(5), e01711-22.

<https://doi.org/10.1128/mbio.01711-22>

- Lagier, J. C., Dubourg, G., Million, M., Cadoret, F., Bilen, M., Fenollar, F., Levasseur, A., Rolain, J. M., Fournier, P. E., & Raoult, D. (2018). Culturing the human microbiota and culturomics. *Nature Reviews Microbiology*, 16(9), 540–550.
<https://doi.org/10.1038/s41579-018-0041-0>
- Lane, D. J. (1991). 16S/23S rRNA sequencing, p. 115-175. In E. Stackebrandt and M. Goodfellow (ed.), *Nucleic acid techniques in bacterial systematics*. John Wiley & Sons, New York.
- Larsson, U., & Hagström, A. (1979). Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. *Marine Biology*, 52(3), 199–206.
<https://doi.org/10.1007/BF00398133>
- Le Chevanton, M., Garnier, M., Bougaran, G., Schreiber, N., Lukomska, E., Bérard, J. B., Fouilland, E., Bernard, O., & Cadoret, J. P. (2013). Screening and selection of growth-promoting bacteria for *Dunaliella* cultures. *Algal Research*, 2(3), 212-222.
<https://doi.org/10.1016/j.algal.2013.05.003>
- Le, V. Van, Ko, S. R., Shin, Y., Kim, K., & Ahn, C. Y. (2024). Succession of particle-attached and free-living bacterial communities in response to microalgal dynamics induced by the biological cyanocide paucibactin A. *Chemosphere*, 358.
<https://doi.org/10.1016/j.chemosphere.2024.142197>
- Lee, J. N., Lee, J. S., Shin, C. S., Park, S. C., & Kim, S. W. (2000). Methods to enhance tolerances of *Chlorella* KR-1 to toxic compounds in flue gas. *Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology*, 84–86, 329–342.
<https://doi.org/10.1385/ABAB:84-86:1-9:329>
- Lee, J. S., Kim, D. K., Lee, J. P., Park, S. C., Koh, J. H., Cho, H. S., & Kim, S. W. (2002). Effects of SO₂ and NO on growth of *Chlorella* sp. KR-1. *Bioresource Technology*, 82(1), 1-4. [https://doi.org/10.1016/S0960-8524\(01\)00158-4](https://doi.org/10.1016/S0960-8524(01)00158-4)
- Lee, K. B., Liu, C. Te, Anzai, Y., Kim, H., Aono, T., & Oyaizu, H. (2005). The hierarchical system of the “Alphaproteobacteria”: Description of Hyphomonadaceae fam. nov., Xanthobacteraceae fam. nov. and Erythrobacteraceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, 55(5), 1907–1919.
<https://doi.org/10.1099/ijs.0.63663-0>
- Lee, Y.-Y., Jonas, L., Hill, R., Place, A., Silsbe, G., Hunsicker, S., North, E., & Li, Y. (2024). Engineering whitening events in culture: A microalgae-driven calcium carbonate and biomass production process at high pH and alkalinity with the marine microalga *Nannochloropsis oceanica* IMET1. *Journal of CO₂ Utilization*, 80, 102669.
<https://doi.org/10.1016/j.jcou.2024.102669>

- Lennon, J. T., Rappuoli, R., Bloom, D. E., Brooke, C., Burckhardt, R. M., Dangour, A. D., Egamberdieva, D., & Gronvall, G. K. (2025). *Microbial solutions for climate change require global partnership*. 6, 9–11. <https://doi.org/10.1128/mbio.00778-25>
- Li, J., Han, D., Wang, D., Ning, K., Jia, J., Wei, L., Jing, X., Huang, S., Chen, J., Li, Y., Hu, Q., & Xu, J. (2014). Choreography of transcriptomes and lipidomes of *Nannochloropsis* reveals the mechanisms of oil synthesis in microalgae. *Plant Cell*, 26(4), 1645–1665. <https://doi.org/10.1105/tpc.113.121418>
- Li, K., Liu, Q., Fang, F., Luo, R., Lu, Q., Zhou, W., Huo, S., Cheng, P., Liu, J., Addy, M., Chen, P., Chen, D., & Ruan, R. (2019). Microalgae-based wastewater treatment for nutrients recovery: A review. *Bioresource Technology*, 291. <https://doi.org/10.1016/j.biortech.2019.121934>
- Li, D., Liu, R., Cui, X., He, M., Zheng, S., Du, W., Gao, M., & Wang, C. (2021). Co-culture of bacteria and microalgae for treatment of high concentration biogas slurry. *Journal of Water Process Engineering*, 41. <https://doi.org/10.1016/j.jwpe.2021.102014>
- Li, J., Wang, Y., Fang, Y., Lyu, X., Zhu, Z., Wu, C., Xu, Z., Li, W., Liu, N., Du, C., & Wang, Y. (2025). Phycospheric bacteria alleviate the stress of erythromycin on *Auxenochlorella pyrenoidosa* by regulating nitrogen metabolism. *Plants*, 14(1), 1–17. <https://doi.org/10.3390/plants14010121>
- Lin, H., Li, Y., & Hill, R. T. (2022). Microalgal and bacterial auxin biosynthesis: Implications for algal biotechnology. *Current Opinion in Biotechnology*, 73, 300-307. <https://doi.org/10.1016/j.copbio.2021.09.006>
- Ling, T., Zhang, Y. F., Cao, J. Y., Xu, J. L., Kong, Z. Y., Zhang, L., Liao, K., Zhou, C. X., & Yan, X. J. (2020). Analysis of bacterial community diversity within seven bait-microalgae. *Algal Research*, 51. <https://doi.org/10.1016/j.algal.2020.102033>
- Lipsman, V., Shlakhter, O., Rocha, J., & Segev, E. (2024). Bacteria contribute exopolysaccharides to an algal-bacterial joint extracellular matrix. *Npj Biofilms and Microbiomes*, 10(1). <https://doi.org/10.1038/s41522-024-00510-y>
- Liu, J., Song, Y., & Qiu, W. (2017). Oleaginous microalgae *Nannochloropsis* as a new model for biofuel production: Review & analysis. *Renewable and Sustainable Energy Reviews*, 72, 154-162. <https://doi.org/10.1016/j.rser.2016.12.120>
- Liu, W., Fan, Y., He, P., & Chen, H. (2021). Complete genome sequence of a nitrate reducing bacteria, *Algoriphagus* sp. Y33 isolated from the water of the Indian Ocean. *Marine Genomics*, 59. <https://doi.org/10.1016/j.margen.2021.100861>
- Liu, X., Wang, K., Wang, J., Zuo, J., Peng, F., Wu, J., & San, E. (2019). Carbon dioxide fixation coupled with ammonium uptake by immobilized *Scenedesmus obliquus* and its potential for

- protein production. *Bioresource Technology*, 289. <https://doi.org/10.1016/j.biortech.2019.121685>
- Liu, Xiaoning, Chen, G., Tao, Y., & Wang, J. (2020). Application of effluent from WWTP in cultivation of four microalgae for nutrients removal and lipid production under the supply of CO₂. *Renewable Energy*, 149, 708–715. <https://doi.org/10.1016/j.renene.2019.12.092>
- Liu, Y. X., Qin, Y., Chen, T., Lu, M., Qian, X., Guo, X., & Bai, Y. (2021). A practical guide to amplicon and metagenomic analysis of microbiome data. *Protein and Cell*, 12(5), 315–330. <https://doi.org/10.1007/s13238-020-00724-8>
- Liu, Z., Zhang, F., & Chen, F. (2013). High throughput screening of CO₂-tolerating microalgae using GasPak bags. *Aquatic Biosystems*, 9(1). <https://doi.org/10.1186/2046-9063-9-23>
- Lloyd, K. G., Steen, A. D., Ladau, J., Yin, J., & Crosby, L. (2018). phylogenetically novel uncultured microbial cells dominate earth microbiomes. *mSystems*, 3(5). <https://doi.org/10.1128/msystems.00055-18>
- Loman, N. J., Quick, J., & Simpson, J. T. (2015). A complete bacterial genome assembled de novo using only nanopore sequencing data. *Nature Methods*, 12(8), 733–735. <https://doi.org/10.1038/nmeth.3444>
- Lubián, L. M., Montero, O., Moreno-Garrido, I., Huertas, I. E., Sobrino, C., González-Del Valle, M., & Parés, G. (2000). *Nannochloropsis* (Eustigmatophyceae) as source of commercially valuable pigments. *Journal of Applied Phycology*, 12(3–5), 733–735. <https://doi.org/10.1023/a:1008170915932>
- Lubzens, E., Gibson, O., Zmora, O., & Sukenik, A. (1995). Potential advantages of frozen algae (*Nannochloropsis* sp.) for rotifer (*Brachionus plicatilis*) culture. *Aquaculture*, 133(3–4), 295–309. [https://doi.org/10.1016/0044-8486\(95\)00010-Y](https://doi.org/10.1016/0044-8486(95)00010-Y)
- Luef, B., Frischkorn, K. R., Wrighton, K. C., Holman, H. Y. N., Birarda, G., Thomas, B. C., Singh, A., Williams, K. H., Siegerist, C. E., Tringe, S. G., Downing, K. H., Comolli, L. R., & Banfield, J. F. (2015). Diverse uncultivated ultra-small bacterial cells in groundwater. *Nature Communications*, 6. <https://doi.org/10.1038/ncomms7372>
- Ma, S., Li, D., Yu, Y., Li, D., Yadav, R. S., & Feng, Y. (2019). Application of a microalga, *Scenedesmus obliquus* PF3, for the biological removal of nitric oxide (NO) and carbon dioxide. *Environmental Pollution*, 252, 344–351. <https://doi.org/10.1016/j.envpol.2019.05.084>
- Ma, S., Yu, Y., Cui, H., Yadav, R. S., Li, J., & Feng, Y. (2020). Unsterilized sewage treatment and carbohydrate accumulation in *Tetradesmus obliquus* PF3 with CO₂ supplementation. *Algal Research*, 45. <https://doi.org/10.1016/j.algal.2019.101741>
- Ma, X. N., Chen, T. P., Yang, B., Liu, J., & Chen, F. (2016). Lipid production from

- Nannochloropsis*. *Marine Drugs*, 14(4). <https://doi.org/10.3390/md14040061>
- Madic, J., Zocevic, A., Senlis, V., Fradet, E., Andre, B., Muller, S., Dangla, R., & Droniou, M. E. (2016). Three-color crystal digital PCR. *Biomolecular Detection and Quantification*, 10, 34-46. <https://doi.org/10.1016/j.bdq.2016.10.002>
- Malla, M. A., Ansari, F. A., Bux, F., & Kumari, S. (2024). Re-vitalizing wastewater: Nutrient recovery and carbon capture through microbe-algae synergy using omics-biology. *Environmental Research*, 259, 119439. <https://doi.org/10.1016/j.envres.2024.119439>
- Marie, D., Partensky, F., Jacquet, S., & Vaulot, D. (1997). Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Applied and Environmental Microbiology*, 63(1), 186 - 193. <https://doi.org/10.1128/aem.63.1.186-193.1997>
- Markewitz, P., Kuckshinrichs, W., Leitner, W., Linssen, J., Zapp, P., Bongartz, R., Schreiber, A., & Müller, T. E. (2012). Worldwide innovations in the development of carbon capture technologies and the utilization of CO₂. *Energy and Environmental Science*, 5(6), 7281-7305. <https://doi.org/10.1039/c2ee03403d>
- Martínez, M. E., Jiménez, J. M., & El Yousfi, F. (1999). Influence of phosphorus concentration and temperature on growth and phosphorus uptake by the microalga *Scenedesmus obliquus*. *Bioresource Technology*, 67(3), 233-240. [https://doi.org/10.1016/S0960-8524\(98\)00120-5](https://doi.org/10.1016/S0960-8524(98)00120-5)
- Martínez, M. E., Sánchez, S., Jiménez, J. M., El Yousfi, F., & Muñoz, L. (2000). Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. *Bioresource Technology*, 73(3), 263-272. [https://doi.org/10.1016/S0960-8524\(99\)00121-2](https://doi.org/10.1016/S0960-8524(99)00121-2)
- Mata, T. M., Melo, A. C., Simões, M., & Caetano, N. S. (2012). Parametric study of a brewery effluent treatment by microalgae *Scenedesmus obliquus*. *Bioresource Technology*, 107, 151-158. <https://doi.org/10.1016/j.biortech.2011.12.109>
- Mayali, X., Franks, P. J. S., & Burton, R. S. (2011). Temporal attachment dynamics by distinct bacterial taxa during a dinoflagellate bloom. *Aquatic Microbial Ecology*, 63(2), 111-122. <https://doi.org/10.3354/ame01483>
- Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P., & Göker, M. (2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*, 14. <https://doi.org/10.1186/1471-2105-14-60>
- Meier-Kolthoff, J. P., Carbasse, J. S., Peinado-Olarte, R. L., & Göker, M. (2022). TYGS and LPSN: A database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Research*, 50, D801-D807. <https://doi.org/10.1093/nar/gkab.902>

- Meza, B., de-Bashan, L. E., & Bashan, Y. (2015). Involvement of indole-3-acetic acid produced by *Azospirillum brasilense* in accumulating intracellular ammonium in *Chlorella vulgaris*. *Research in Microbiology*, 166(2), 72-83. <https://doi.org/10.1016/j.resmic.2014.12.010>
- Miyachi, S., Iwasaki, I., & Shiraiwa, Y. (2003). Historical perspective on microalgal and cyanobacterial acclimation to low- and extremely high-CO₂ conditions. *Photosynthesis Research*, 77(2-3), 139–153. <https://doi.org/10.1023/A:1025817616865>
- Moghimifam, R., Niknam, V., Ebrahimzadeh, H., & Hejazi, M. A. (2020). CO₂ biofixation and fatty acid composition of two indigenous *Dunaliella* sp. isolates (ABRIINW-CH2 and ABRIINW-SH33) in response to extremely high CO₂ levels. *Bioprocess and Biosystems Engineering*, 43(9), 1587–1597. <https://doi.org/10.1007/s00449-020-02350-4>
- Møller Jensen, L. (1983). Phytoplankton release of extracellular organic carbon, molecular weight composition, and bacterial assimilation. *Marine Ecology Progress Series*, 11, 39-48. <https://doi.org/10.3354/meps011039>
- Moreira, D., Zivanovic, Y., López-Archilla, A. I., Iniesto, M., & López-García, P. (2021). Reductive evolution and unique predatory mode in the CPR bacterium *Vampirococcus lugosii*. *Nature Communications*, 12(1). <https://doi.org/10.1038/s41467-021-22762-4>
- Morley, A. A. (2014). Digital PCR: A brief history. *Biomolecular Detection and Quantification*, 1(1), 1-2. <https://doi.org/10.1016/j.bdq.2014.06.001>
- Mousavi, S. A., Österman, J., Wahlberg, N., Nesme, X., Lavire, C., Vial, L., Paulin, L., de Lajudie, P., & Lindström, K. (2014). Phylogeny of the *Rhizobium-Allorhizobium-Agrobacterium* clade supports the delineation of *Neorhizobium* gen. nov. *Systematic and Applied Microbiology*, 37(3), 208-215. <https://doi.org/10.1016/j.syapm.2013.12.007>
- Mühlenbruch, M., Grossart, H. P., Eigemann, F., & Voss, M. (2018). Mini-review: Phytoplankton-derived polysaccharides in the marine environment and their interactions with heterotrophic bacteria. *Environmental Microbiology*, 20(8), 2671-2685. <https://doi.org/10.1111/1462-2920.14302>
- Murray, A. E., Freudenstein, J., Gribaldo, S., Hatzenpichler, R., Hugenholtz, P., Kämpfer, P., Konstantinidis, K. T., Lane, C. E., Papke, R. T., Parks, D. H., Rossello-Mora, R., Stott, M. B., Sutcliffe, I. C., Thrash, J. C., Venter, S. N., Whitman, W. B., Acinas, S. G., Amann, R. I., Anantharaman, K., ... Reysenbach, A. L. (2020). Roadmap for naming uncultivated Archaea and Bacteria. *Nature Microbiology*, 5(8), 2671-2685. <https://doi.org/10.1038/s41564-020-0733-x>
- Myklestad, S. M. (1995). Release of extracellular products by phytoplankton with special emphasis on polysaccharides. *Science of the Total Environment*, 165(1–3), 155-164. [https://doi.org/10.1016/0048-9697\(95\)04549-G](https://doi.org/10.1016/0048-9697(95)04549-G)
- Naveed, S., Li, C., Lu, X., Chen, S., Yin, B., Zhang, C., & Ge, Y. (2019). Microalgal

- extracellular polymeric substances and their interactions with metal(loid)s: A review. *Critical Reviews in Environmental Science and Technology*, 49(19).
<https://doi.org/10.1080/10643389.2019.1583052>
- Nedelcu, A. M., Lee, R. W., Lemieux, C., Gray, M. W., & Burger, G. (2000). The complete mitochondrial DNA sequence of *Scenedesmus obliquus* reflects an intermediate stage in the evolution of the green algal mitochondrial genome. *Genome Research*, 10(6), 819–831.
<https://doi.org/10.1101/gr.10.6.819>
- Negoro, M., Hamasaki, A., Ikuta, Y., Makita, T., Hirayama, K., & Suzuki, S. (1993). Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. *Applied Biochemistry and Biotechnology*, 39–40(1), 643–653.
<https://doi.org/10.1007/BF02919025>
- Nelson, W. C., & Stegen, J. C. (2015). The reduced genomes of *Parcubacteria* (OD1) contain signatures of a symbiotic lifestyle. *Frontiers in Microbiology*, 6.
<https://doi.org/10.3389/fmicb.2015.00713>
- Novoveská, L., Nielsen, S. L., Eroldoğan, O. T., Haznedaroglu, B. Z., Rinkevich, B., Fazi, S., Robbens, J., Vasquez, M., & Einarsson, H. (2023). Overview and challenges of large-scale cultivation of photosynthetic microalgae and cyanobacteria. *Marine Drugs*, 21(8).
<https://doi.org/10.3390/md21080445>
- Obernosterer, I., & Herndl, G. J. (1995). Phytoplankton extracellular release and bacterial growth: Dependence on the inorganic N:P ratio. *Marine Ecology Progress Series*, 116(1–3), 247–257. <https://doi.org/10.3354/meps116247>
- Oliveira, C. Y. B. d., Oliveira, C. D. L., Prasad, R., Ong, H. C., Araujo, E. S., Shabnam, N., & Gálvez, A. O. (2021). A multidisciplinary review of *Tetradismus obliquus*: a microalga suitable for large-scale biomass production and emerging environmental applications. *Reviews in Aquaculture*, 13(3), 1594–1618. <https://doi.org/10.1111/raq.12536>
- Oliveira, C. Y. B. d, Viegas, T. L., da Silva, M. F. O., Fracalossi, D. M., Lopes, R. G., & Derner, R. B. (2020). Effect of trace metals on growth performance and accumulation of lipids, proteins, and carbohydrates on the green microalga *Scenedesmus obliquus*. *Aquaculture International*, 28(4), 1435–1444. <https://doi.org/10.1007/s10499-020-00533-0>
- Olson, R. D., Assaf, R., Brettin, T., Conrad, N., Cucinell, C., Davis, J. J., Dempsey, D. M., Dickerman, A., Dietrich, E. M., Kenyon, R. W., Kuscuoglu, M., Lefkowitz, E. J., Lu, J., Machi, D., Macken, C., Mao, C., Niewiadomska, A., Nguyen, M., Olsen, G. J., ... Stevens, R. L. (2023). Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): a resource combining PATRIC, IRD and ViPR. *Nucleic Acids Research*, 51(1), D678–D689. <https://doi.org/10.1093/nar/gkac1003>
- Ono, E., & Cuello, J. L. (2007). Carbon dioxide mitigation using thermophilic cyanobacteria. *biosystems engineering*, 96(1), 129–134.

<https://doi.org/10.1016/J.BIOSYSTEMSENG.2006.09.010>

- Oren, A., Da Costa, M. S., Garrity, G. M., Rainey, F. A., Rosselló-Móra, R., Schink, B., Sutcliffe, I., Trujillo, M. E., & Whitman, W. B. (2015). Proposal to include the rank of phylum in the international code of nomenclature of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, 65(11). <https://doi.org/10.1099/ijsem.0.000664>
- Oren, A. (2017). A plea for linguistic accuracy – also for Candidatus taxa. *International Journal of Systematic and Evolutionary Microbiology*, 67(4), 129–134. <https://doi.org/10.1099/ijsem.0.001715>
- Oren, A., Arahal, D. R., Göker, M., Moore, E. R. B., Rossello-Mora, R., & Sutcliffe, I. C. (2023). International Code of Nomenclature of Prokaryotes. Prokaryotic Code (2022 Revision). *International Journal of Systematic and Evolutionary Microbiology*, 73(5). <https://doi.org/10.1099/ijsem.0.005585>
- Overbeek, R., Olson, R., Pusch, G. D., Olsen, G. J., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A. R., Xia, F., & Stevens, R. (2014). The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Research*, 42(D1), D206–D214. <https://doi.org/10.1093/nar/gkt1226>
- Ozkan, A., Kinney, K., Katz, L., & Berberoglu, H. (2012). Reduction of water and energy requirement of algae cultivation using an algae biofilm photobioreactor. *Bioresource Technology*, 114, 542–548. <https://doi.org/10.1016/j.biortech.2012.03.055>
- Packer, M. (2009). Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy. *Energy Policy*, 37(9). <https://doi.org/10.1016/j.enpol.2008.12.025>
- Paerl, H. (1996). Microscale physiological and ecological studies of aquatic cyanobacteria: Macroscale implications. *Microscopy & Research Technique*, 33(1): 47–72. [https://doi.org/10.1002/\(SICI\)1097-0029\(199601\)33:1<47::AID-JEMT6>3.0.CO;2-Y](https://doi.org/10.1002/(SICI)1097-0029(199601)33:1<47::AID-JEMT6>3.0.CO;2-Y)
- Pagnussat, L. A., Maroniche, G., Curatti, L., & Creus, C. (2020). Auxin-dependent alleviation of oxidative stress and growth promotion of *Scenedesmus obliquus* C1S by *Azospirillum brasilense*. *Algal Research*, 47, 3428–3437. <https://doi.org/10.1016/j.algal.2020.101839>
- Palacios, O. A., López, B. R., & de-Bashan, L. E. (2022). Microalga Growth-Promoting Bacteria (MGPB): A formal term proposed for beneficial bacteria involved in microalgal–bacterial interactions. *Algal Research*, 61. <https://doi.org/10.1016/j.algal.2021.102585>
- Pan, K., Qin, J., Li, S., Dai, W., Zhu, B., Jin, Y., Yu, W., Yang, G., & Li, D. (2011). Nuclear monoploidy and asexual propagation of *Nannochloropsis oceanica* (eustigmatophyceae) as revealed by its genome sequence. *Journal of Phycology*, 47(6). <https://doi.org/10.1111/j.1529-8817.2011.01057.x>

- Park, J., Park, B. S., Wang, P., Patidar, S. K., Kim, J. H., Kim, S. H., & Han, M. S. (2017). Phycospheric native bacteria *Pelagibaca bermudensis* and *Stappia* sp. ameliorate biomass productivity of *Tetraselmis striata* (KCTC1432BP) in co-cultivation system through mutualistic interaction. *Frontiers in Plant Science*, 8, 1425-1432. <https://doi.org/10.3389/fpls.2017.00289>
- Parker, C. T., Tindall, B. J., & Garrity, G. M. (2019). International code of nomenclature of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, 69(1). <https://doi.org/10.1099/ijsem.0.000778>
- Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., & Tyson, G. W. (2015). CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research*, 25(7), D785–D794. <https://doi.org/10.1101/gr.186072.114>
- Parks, D. H., Rinke, C., Chuvochina, M., Chaumeil, P. A., Woodcroft, B. J., Evans, P. N., Hugenholtz, P., & Tyson, G. W. (2017). Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. *Nature Microbiology*, 2(11), D785–D794. <https://doi.org/10.1038/s41564-017-0012-7>
- Parks, D. H., Chuvochina, M., Waite, D. W., Rinke, C., Skarshewski, A., Chaumeil, P. A., & Hugenholtz, P. (2018). A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nature Biotechnology*, 36(10). <https://doi.org/10.1038/nbt.4229>
- Parks, D. H., Chuvochina, M., Rinke, C., Mussig, A. J., Chaumeil, P. A., & Hugenholtz, P. (2022). GTDB: An ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. *Nucleic Acids Research*, 50(1), D785–D794. <https://doi.org/10.1093/nar/gkab776>
- Parrello, B., Butler, R., Chlenski, P., Olson, R., Overbeek, J., Pusch, G. D., Vonstein, V., & Overbeek, R. (2019). A machine learning-based service for estimating quality of genomes using PATRIC. *BMC Bioinformatics*, 20(1). <https://doi.org/10.1186/s12859-019-3068-y>
- Patz, S., Gautam, A., Becker, M., Ruppel, S., Rodríguez-Palenzuela, P., & Huson, D. (2021). PLABase: A comprehensive web resource for analyzing the plant growth-promoting potential of plant-associated bacteria. *BioRxiv*, 3. <https://www.biorxiv.org/content/10.1101/2021.12.13.472471v1%0Ahttps://www.biorxiv.org/content/10.1101/2021.12.13.472471v1.abstract>
- Peixoto, R., Voolstra, C. R., Stein, L. Y., Hugenholtz, P., Salles, J. F., Amin, S. A., Häggblom, M., Gregory, A., Makhalanyane, T. P., Wang, F., Agbodjato, N. A., Wang, Y., Jiao, N., Lennon, J. T., Ventosa, A., Bavoil, P. M., Miller, V., & Gilbert, J. A. (2024). Microbial solutions must be deployed against climate catastrophe. *Nature Reviews Microbiology*, 23, 1-2. <https://doi.org/10.1038/s41579-024-01123-0>

- Peng, H., de-Bashan, L. E., Bashan, Y., & Higgins, B. T. (2020). Indole-3-acetic acid from *Azospirillum brasilense* promotes growth in green algae at the expense of energy storage products. *Algal Research*, 47. <https://doi.org/10.1016/j.algal.2020.101845>
- Peng, H., de-Bashan, L. E., & Higgins, B. T. (2021). Comparison of algae growth and symbiotic mechanisms in the presence of plant growth promoting bacteria and non-plant growth promoting bacteria. *Algal Research*, 53. <https://doi.org/10.1016/j.algal.2020.102156>
- Piontek, J., Händel, N., De Bodt, C., Harlay, J., Chou, L., & Engel, A. (2011). The utilization of polysaccharides by heterotrophic bacterioplankton in the Bay of Biscay (North Atlantic Ocean). *Journal of Plankton Research*, 33(11). <https://doi.org/10.1093/plankt/fbr069>
- Powell, R. J. (2014). Rapid harvest of algae for biofuel production with the aggregating bacterium *Bacillus* sp. strain rp1137. University of Maryland Center for Environmental Science.
- Price, M. N., Deutschbauer, A. M., & Arkin, A. P. (2020). GapMind: Automated annotation of amino acid biosynthesis. *mSystems*, 5(3). <https://doi.org/10.1128/msystems.00291-20>
- Probst, A. J., Ladd, B., Jarett, J. K., Geller-Mcgrath, D. E., Sieber, C. M. K., Emerson, J. B., Anantharaman, K., Thomas, B. C., Malmstrom, R. R., Stieglmeier, M., Klingl, A., Woyke, T., Ryan, M. C., & Banfield, J. F. (2018). Differential depth distribution of microbial function and putative symbionts through sediment-hosted aquifers in the deep terrestrial subsurface. *Nature Microbiology*, 3(3), D785–D794. <https://doi.org/10.1038/s41564-017-0098-y>
- Proctor, C. R., Besmer, M. D., Langenegger, T., Beck, K., Walser, J. C., Ackermann, M., Bürgmann, H., & Hammes, F. (2018). Phylogenetic clustering of small low nucleic acid-content bacteria across diverse freshwater ecosystems. *ISME Journal*, 12(5), 1344–1359. <https://doi.org/10.1038/s41396-018-0070-8>
- Radakovits, R., Jinkerson, R. E., Fuerstenberg, S. I., Tae, H., Settlege, R. E., Boore, J. L., & Posewitz, M. C. (2012). Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropsis gaditana*. *Nature Communications*, 3. <https://doi.org/10.1038/ncomms1688>
- Rahaman, M. S. A., Cheng, L. H., Xu, X. H., Zhang, L., & Chen, H. L. (2011). A review of carbon dioxide capture and utilization by membrane integrated microalgal cultivation processes. *Renewable and Sustainable Energy Reviews*, 15(8), 4002–4012. <https://doi.org/10.1016/j.rser.2011.07.031>
- Rahlff, J., Giebel, H. A., Stolle, C., Wurl, O., Probst, A. J., & Herlemann, D. P. R. (2020). Overlooked diversity of ultramicrobacterial minorities at the air-sea interface. *Atmosphere*, 11(11). <https://doi.org/10.3390/atmos1111214>

- Ramanan, R., Kang, Z., Kim, B. H., Cho, D. H., Jin, L., Oh, H. M., & Kim, H. S. (2015). Phycosphere bacterial diversity in green algae reveals an apparent similarity across habitats. *Algal Research*, 8, 140-144. <https://doi.org/10.1016/j.algal.2015.02.003>
- Ramanan, R., Kim, B. H., Cho, D. H., Oh, H. M., & Kim, H. S. (2016). Algae-bacteria interactions: Evolution, ecology and emerging applications. *Biotechnology Advances*, 34(1), 14–29. <https://doi.org/10.1016/j.biotechadv.2015.12.003>
- Ramonedá, J., Jensen, T. B. N., Price, M. N., Casamayor, E. O., & Fierer, N. (2023). Taxonomic and environmental distribution of bacterial amino acid auxotrophies. *Nature Communications*, 14(1). <https://doi.org/10.1038/s41467-023-43435-4>
- Rao, M., Zou, X., Ye, J., Kuang, C., Chen, C., Huang, C., Chen, G., Qin, S., Wang, Z., Feng, L., Tang, Y., Tian, J., & Cheng, J. (2022). Light conditions determine optimal CO₂ concentrations for *Nannochloropsis oceanica* growth with carbon fixation. *ACS Sustainable Chemistry and Engineering*, 10(27), 8799–8814. <https://doi.org/10.1021/acssuschemeng.2c01179>
- Rappé, M. S., & Giovannoni, S. J. (2003). The uncultured microbial majority. *Annual Review of Microbiology*, 57, 369-394. <https://doi.org/10.1146/annurev.micro.57.030502.090759>
- Rawat, D., Sharma, U., Poria, P., Finlan, A., Parker, B., Sharma, R. S., & Mishra, V. (2022). Iron-dependent mutualism between *Chlorella sorokiniana* and *Ralstonia pickettii* forms the basis for a sustainable bioremediation system. *ISME Communications*, 2(1). <https://doi.org/10.1038/s43705-022-00161-0>
- Razzak, S. A., Ilyas, M., Ali, S. A. M., & Hossain, M. M. (2015). Effects of CO₂ concentration and pH on mixotrophic growth of *Nannochloropsis oculata*. *Applied Biochemistry and Biotechnology*, 176(5), 1290–1302. <https://doi.org/10.1007/s12010-015-1646-7>
- Richter, M., & Rosselló-Móra, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proceedings of the National Academy of Sciences of the United States of America*, 106(45), 19126-19131. <https://doi.org/10.1073/pnas.0906412106>
- Riemann, L., Steward, G. F., & Azam, F. (2000). Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Applied and Environmental Microbiology*, 66(2), 578 - 587. <https://doi.org/10.1128/AEM.66.2.578-587.2000>
- Riesco, R., & Trujillo, M. E. (2024). Update on the proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, 74(3), 1–12. <https://doi.org/10.1099/ijsem.0.006300>
- Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N. N., Anderson, I. J., Cheng, J. F., Darling, A., Malfatti, S., Swan, B. K., Gies, E. A., Dodsworth, J. A., Hedlund, B. P., Tsiamis, G., Sievert, S. M., Liu, W. T., Eisen, J. A., Hallam, S. J., Kyrpides, N. C., Stepanauskas, R., ... Woyke, T. (2013). Insights into the phylogeny and coding potential of microbial dark

- matter. *Nature*, 499(7459), 431–437. <https://doi.org/10.1038/nature12352>
- Robinson, J. T., Thorvaldsdottir, H., Turner, D., & Mesirov, J. P. (2023). Igv.js: an embeddable JavaScript implementation of the Integrative Genomics Viewer (IGV). *Bioinformatics*, 39(1). <https://doi.org/10.1093/bioinformatics/btac830>
- Rodas-Zuluaga, L. I., Castañeda-Hernández, L., Castillo-Vacas, E. I., Gradiz-Menjivar, A., López-Pacheco, I. Y., Castillo-Zacarias, C., Bouilly, L., Iqbal, H. M. N., & Parra-Saldívar, R. (2021). Bio-capture and influence of CO₂ on the growth rate and biomass composition of the microalgae *Botryococcus braunii* and *Scenedesmus* sp. *Journal of CO₂ Utilization*, 43. <https://doi.org/10.1016/j.jcou.2020.101371>
- Rodolfi, L., Zittelli, G. C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., & Tredici, M. R. (2009). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering*, 102(1), 100–112. <https://doi.org/10.1002/biot.22033>
- Rodriguez-R, L. M., Gunturu, S., Harvey, W. T., Rosselló-Mora, R., Tiedje, J. M., Cole, J. R., & Konstantinidis, K. T. (2018). The Microbial Genomes Atlas (MiGA) webserver: Taxonomic and gene diversity analysis of Archaea and Bacteria at the whole genome level. *Nucleic Acids Research*, 46(1), W282–W288. <https://doi.org/10.1093/nar/gky467>
- Rooney-Varga, J. N., Giewat, M. W., Savin, M. C., Sood, S., Legresley, M., & Martin, J. L. (2005). Links between phytoplankton and bacterial community dynamics in a coastal marine environment. *Microbial Ecology*, 49(1), 163–175. <https://doi.org/10.1007/s00248-003-1057-0>
- Ruiz-Marin, A., Mendoza-Espinosa, L. G., & Stephenson, T. (2010). Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology*, 101(1), 58–64. <https://doi.org/10.1016/j.biortech.2009.02.076>
- Ruiz, J., Álvarez, P., Arbib, Z., Garrido, C., Barragán, J., & Perales, J. A. (2011). Effect of nitrogen and phosphorus concentration on their removal kinetic in treated urban wastewater by *Chlorella vulgaris*. *International Journal of Phytoremediation*, 13(9). <https://doi.org/10.1080/15226514.2011.573823>
- Ruiz, J., Arbib, Z., Álvarez-Díaz, P. D., Garrido-Pérez, C., Barragán, J., & Perales, J. A. (2014). Influence of light presence and biomass concentration on nutrient kinetic removal from urban wastewater by *Scenedesmus obliquus*. *Journal of Biotechnology*, 178(1), 32–37. <https://doi.org/10.1016/j.jbiotec.2014.03.001>
- Salas, A., Cabrera, J. J., Jiménez-Leiva, A., Mesa, S., Bedmar, E. J., Richardson, D. J., Gates, A. J., & Delgado, M. J. (2021). Bacterial nitric oxide metabolism: Recent insights in rhizobia. *Advances in Microbial Physiology*, 78, 259–315. <https://doi.org/10.1016/bs.ampbs.2021.05.001>

- Salih, F. M. (2011). Microalgae tolerance to high concentrations of carbon dioxide: A Review. *Journal of Environmental Protection*, 2(5), 648-654. <https://doi.org/10.4236/jep.2011.25074>
- Samo, T. J., Kimbrel, J. A., Nilson, D. J., Pett-Ridge, J., Weber, P. K., & Mayali, X. (2018). Attachment between heterotrophic bacteria and microalgae influences symbiotic microscale interactions. *Environmental Microbiology*, 20(12), 4385-4400. <https://doi.org/10.1111/1462-2920.14357>
- Sánchez, C., & Minamisawa, K. (2018). Redundant roles of *Bradyrhizobium oligotrophicum* Cu-type (NirK) and cd1-type (NirS) nitrite reductase genes under denitrifying conditions. *FEMS Microbiology Letters*, 365 (5). <https://doi.org/10.1093/femsle/fny015>
- Sandnes, J. M., Källqvist, T., Wenner, D., & Gislerød, H. R. (2005). Combined influence of light and temperature on growth rates of *Nannochloropsis oceanica*: Linking cellular responses to large-scale biomass production. *Journal of Applied Phycology*, 17(6), 515–525. <https://doi.org/10.1007/s10811-005-9002-x>
- Sapp, M., Schwaderer, A. S., Wiltshire, K. H., Hoppe, H. G., Gerds, G., & Wichels, A. (2007). Species-specific bacterial communities in the phycosphere of microalgae? *Microbial Ecology*, 53(4), 683–699. <https://doi.org/10.1007/s00248-006-9162-5>
- Savvidou, M. G., Ferraro, A., Schinas, P., Mamma, D., Kekos, D., Hristoforou, E., & Kolisis, F. N. (2022). Magnetic immobilization and growth of *Nannochloropsis oceanica* and *Senedasmus almeriensis*. *Plants*, 11(1). <https://doi.org/10.3390/plants11010072>
- Schmeisser, C., Krohn-Molt, I., & Streit, W. R. (2017). Metagenome analyses of multispecies microbial biofilms: First steps toward understanding diverse microbial systems on surfaces. *Functional Metagenomics: Tools and Applications*, 201–215. https://doi.org/10.1007/978-3-319-61510-3_12
- Schnoes, A. M., Brown, S. D., Dodevski, I., & Babbitt, P. C. (2009). Annotation error in public databases: Misannotation of molecular function in enzyme superfamilies. *PLoS Computational Biology*, 5(12), e1000605. <https://doi.org/10.1371/journal.pcbi.1000605>
- Schulz, F., Eloë-Fadrosch, E. A., Bowers, R. M., Jarett, J., Nielsen, T., Ivanova, N. N., Kyrpides, N. C., & Woyke, T. (2017). Towards a balanced view of the bacterial tree of life. *Microbiome*, 5(1). <https://doi.org/10.1186/s40168-017-0360-9>
- Segev, E., Wyche, T. P., Kim, K. H., Petersen, J., Ellebrandt, C., Vlamakis, H., Barteneva, N., Paulson, J. N., Chai, L., Clardy, J., & Kolter, R. (2016). Dynamic metabolic exchange governs a marine algal-bacterial interaction. *eLife*, 5. <https://doi.org/10.7554/eLife.17473>
- Seyedsayamdost, M. R., Carr, G., Kolter, R., & Clardy, J. (2011). Roseobacticides: Small molecule modulators of an algal-bacterial symbiosis. *Journal of the American Chemical Society*, 133(45), 18343–18349. <https://doi.org/10.1021/ja207172s>

- Seymour, J. R., Amin, S. A., Raina, J. B., & Stocker, R. (2017). Zooming in on the phycosphere: The ecological interface for phytoplankton-bacteria relationships. *Nature Microbiology*, 2. <https://doi.org/10.1038/nmicrobiol.2017.65>
- Sforza, E., Gris, B., De Farias Silva, C. E., Morosinotto, T., & Bertucco, A. (2014). Effects of light on cultivation of *Scenedesmus obliquus* in batch and continuous flat plate photobioreactor. *Chemical Engineering Transactions*, 38, 211-216. <https://doi.org/10.3303/CET1438036>
- Shen, Q. H., Gong, Y. P., Fang, W. Z., Bi, Z. C., Cheng, L. H., Xu, X. H., & Chen, H. L. (2015). Saline wastewater treatment by *Chlorella vulgaris* with simultaneous algal lipid accumulation triggered by nitrate deficiency. *Bioresource Technology*, 193, 68-75. <https://doi.org/10.1016/j.biortech.2015.06.050>
- Shi, Y., Ji, B., Li, A., Zhang, X., & Liu, Y. (2024). Enhancing the performance of microalgal-bacterial systems with sodium bicarbonate: A step forward to carbon neutrality of municipal wastewater treatment. *Water Research*, 266. <https://doi.org/10.1016/j.watres.2024.122345>
- Singh, H. M., Kothari, R., Gupta, R., & Tyagi, V. V. (2019). Bio-fixation of flue gas from thermal power plants with algal biomass: Overview and research perspectives. *Journal of Environmental Management*, 245, 519-539. <https://doi.org/10.1016/j.jenvman.2019.01.043>
- Singh, S. K., Major, S. R., Cai, H., Chen, F., Hill, R. T., & Li, Y. (2018). Draft genome sequences of *Cloacibacterium normanense* IMET F, a microalgal growth-promoting bacterium, and *Aeromonas jandaei* IMET J, a microalgal growth-inhibiting bacterium. *Genome Announcements*, 6(24). <https://doi.org/10.1128/genomeA.00503-18>
- Skorupa, D. J., Akyel, A., Fields, M. W., & Gerlach, R. (2019). Facultative and anaerobic consortia of haloalkaliphilic ureolytic micro-organisms capable of precipitating calcium carbonate. *Journal of Applied Microbiology*, 127(5), 1479–1489. <https://doi.org/10.1111/jam.14384>
- Smith, D. C., Steward, G. F., Long, R. A., & Azam, F. (1995). Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. *Deep-Sea Research Part II*, 42(1), 75-97. [https://doi.org/10.1016/0967-0645\(95\)00005-B](https://doi.org/10.1016/0967-0645(95)00005-B)
- Smith, G. M. (1913). *Tetradismus*, a new four-celled coenobitic alga. *Bulletin of the Torrey Botanical Club*, 40(2), 75-87. <https://doi.org/10.2307/2479812>
- Smriga, S., Fernandez, V. I., Mitchell, J. G., & Stocker, R. (2016). Chemotaxis toward phytoplankton drives organic matter partitioning among marine bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 113(6), 1576-1581. <https://doi.org/10.1073/pnas.1512307113>
- Sonowal, S., Ahmed, R., Chikkaputtaiah, C., Basar, E., & Velmurugan, N. (2022). A

- comprehensive characterization of culturable endophytic bacteria of *Paris polyphylla* and their potential role in microalgal growth in co-culture. *Applied Soil Ecology*, 174. <https://doi.org/10.1016/j.apsoil.2022.104410>
- Sonowal, S., Palani, N. P., Ahmed, R., Debbarma, J., Chikkaputtaiah, C., Basar, E., & Velmurugan, N. (2022). Himalayan bacterial endophytes enhance microalgal cell numbers and chlorophyll content in synthetic co-culture. *Journal of Applied Phycology*, 34(5), 1576-1581. <https://doi.org/10.1007/s10811-022-02798-9>
- Speeckaert, G., Borges, A. V., & Gypens, N. (2019). Salinity and growth effects on dimethylsulfoniopropionate (DMSP) and dimethylsulfoxide (DMSO) cell quotas of *Skeletonema costatum*, *Phaeocystis globosa* and *Heterocapsa triquetra*. *Estuarine, Coastal and Shelf Science*, 226. <https://doi.org/10.1016/j.ecss.2019.106275>
- Srinivas, P., Peterson, S. B., Gallagher, L. A., Wang, Y., & Mougous, J. D. (2024). Beyond genomics in Patescibacteria: A trove of unexplored biology packed into ultrasmall bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 121(51), 1–7. <https://doi.org/10.1073/pnas.2419369121>
- Stackebrandt, E., & Liesack, W. (1993). Nucleic acid and classification. In M. Goodfellow (Ed.), *The New Bacterial Systematics* (pp. 151-194). London: Academic Press.
- Staley, J. T., & Konopka, A. (1985). Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annual Review of Microbiology*, 39. <https://doi.org/10.1146/annurev.mi.39.100185.001541>
- Steichen, S. A., Gao, S., Waller, P., & Brown, J. K. (2020). Association between algal productivity and phycosphere composition in an outdoor *Chlorella sorokiniana* reactor based on multiple longitudinal analyses. *Microbial Biotechnology*, 13(5), 321-346. <https://doi.org/10.1111/1751-7915.13591>
- Suda, S., Atsumi, M., & Miyashita, H. (2002). Taxonomic characterization of a marine *Nannochloropsis* species, *N. oceanica* sp. nov. (Eustigmatophyceae). *Phycologia*, 41(3), 273-279. <https://doi.org/10.2216/i0031-8884-41-3-273.1>
- Sumrall, J. B., Larson, E. B., & Mylroie, J. E. (2017). Very high magnesium calcite formation and microbial communities found in porosity of the Seroe Domi Formation of Curacao, Netherland Antilles. *Carbonates and Evaporites*, 32(2), 123–133. <https://doi.org/10.1007/s13146-017-0352-7>
- Sun, L., Bai, Z., Yang, Q., Fu, R., Li, H., & Li, X. (2024). In situ assessment of the initial phase of wastewater biofilm formation: Effect of the presence of algae in an aerobic bacterial biofilm system. *Water Research*, 253. <https://doi.org/10.1016/j.watres.2024.121283>
- Sutcliffe, I. C., Rosselló-Móra, R., & Trujillo, M. E. (2021). Addressing the sublime scale of the microbial world: reconciling an appreciation of microbial diversity with the need to describe

- species. *New Microbes and New Infections*, 43. <https://doi.org/10.1016/j.nmni.2021.100931>
- Sutcliffe, I. C., Dijkshoorn, L., & Whitman, W. B. (2020). Minutes of the international committee on systematics of prokaryotes online discussion on the proposed use of gene sequences as type for naming of prokaryotes, and outcome of vote. *International Journal of Systematic and Evolutionary Microbiology*, 70(7). <https://doi.org/10.1099/ijsem.0.004303>
- Suthers, P. F., Dasika, M. S., Kumar, V. S., Denisov, G., Glass, J. I., & Maranas, C. D. (2009). Genome-scale metabolic reconstruction of *Mycoplasma genitalium*, iPS189. *PLoS Computational Biology*, 5(2). <https://doi.org/10.1371/journal.pcbi.1000285>
- Takahashi, T. (2019). Routine management of microalgae using autofluorescence from chlorophyll. *Molecules*, 24(24). <https://doi.org/10.3390/molecules24244441>
- Tang, D., Han, W., Li, P., Miao, X., & Zhong, J. (2011). CO₂ biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO₂ levels. *Bioresource Technology*, 102(3), 3071-3076. <https://doi.org/10.1016/j.biortech.2010.10.047>
- Thornton, D. C. O. (2014). Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean. *European Journal of Phycology*, 49(1), 20-46. <https://doi.org/10.1080/09670262.2013.875596>
- Thorvaldsdóttir, H., Robinson, J. T., & Mesirov, J. P. (2013). Integrative Genomics Viewer (IGV): High-performance genomics data visualization and exploration. *Briefings in Bioinformatics*, 14(2), 178–192. <https://doi.org/10.1093/bib/bbs017>
- Tian, R., Ning, D., He, Z., Zhang, P., Spencer, S. J., Gao, S., Shi, W., Wu, L., Zhang, Y., Yang, Y., Adams, B. G., Rocha, A. M., Detienne, B. L., Lowe, K. A., Joyner, D. C., Klingeman, D. M., Arkin, A. P., Fields, M. W., Hazen, T. C., ... Zhou, J. (2020). Small and mighty: Adaptation of superphylum Patescibacteria to groundwater environment drives their genome simplicity. *Microbiome*, 8(1). <https://doi.org/10.1186/s40168-020-00825-w>
- Tong, C. Y., Honda, K., & Derek, C. J. C. (2023). A review on microalgal-bacterial co-culture: The multifaceted role of beneficial bacteria towards enhancement of microalgal metabolite production. *Environmental Research*, 228. <https://doi.org/10.1016/j.envres.2023.115872>
- Toyama, T., Kasuya, M., Hanaoka, T., Kobayashi, N., Tanaka, Y., Inoue, D., Sei, K., Morikawa, M., & Mori, K. (2018). Growth promotion of three microalgae, *Chlamydomonas reinhardtii*, *Chlorella vulgaris* and *Euglena gracilis*, by *in situ* indigenous bacteria in wastewater effluent. *Biotechnology for Biofuels*, 11(1). <https://doi.org/10.1186/s13068-018-1174-0>
- Van Vooren, G., Le Grand, F., Legrand, J., Cuiné, S., Peltier, G., & Pruvost, J. (2012). Investigation of fatty acids accumulation in *Nannochloropsis oculata* for biodiesel application. *Bioresource Technology*, 124, 421-432.

<https://doi.org/10.1016/j.biortech.2012.08.009>

- Vavourakis, C. D., Ghai, R., Rodriguez-Valera, F., Sorokin, D. Y., Tringe, S. G., Hugenholtz, P., & Muyzer, G. (2016). Metagenomic insights into the uncultured diversity and physiology of microbes in four hypersaline soda lake brines. *Frontiers in Microbiology*, 7. <https://doi.org/10.3389/fmicb.2016.00211>
- Veldhuis, M. J. W., Admiraal, W., & Colijn, F. (1986). Chemical and physiological changes of phytoplankton during the spring bloom, dominated by *Phaeocystis pouchetii* (Haptophyceae): Observations in Dutch coastal waters of the North Sea. *Netherlands Journal of Sea Research*, 20(1), 49-60. [https://doi.org/10.1016/0077-7579\(86\)90060-8](https://doi.org/10.1016/0077-7579(86)90060-8)
- Venancio, H. C., Cella, H., Lopes, R. G., & Derner, R. B. (2020). Surface-to-volume ratio influence on the growth of *Scenedesmus obliquus* in a thin-layer cascade system. *Journal of Applied Phycology*, 32(2), 821–829. <https://doi.org/10.1007/s10811-020-02036-0>
- Vendruscolo, R. G., Fagundes, M. B., Maroneze, M. M., do Nascimento, T. C., de Menezes, C. R., Barin, J. S., Zepka, L. Q., Jacob-Lopes, E., & Wagner, R. (2019). *Scenedesmus obliquus* metabolomics: effect of photoperiods and cell growth phases. *Bioprocess and Biosystems Engineering*, 42(5), 727–739. <https://doi.org/10.1007/s00449-019-02076-y>
- Vieler, A., Wu, G., Tsai, C. H., Bullard, B., Cornish, A. J., Harvey, C., Reca, I. B., Thornburg, C., Achawanantakun, R., Buehl, C. J., Campbell, M. S., Cavalier, D., Childs, K. L., Clark, T. J., Deshpande, R., Erickson, E., Armenia Ferguson, A., Handee, W., Kong, Q., ... Benning, C. (2012). Genome, functional gene annotation, and nuclear transformation of the heterokont oleaginous alga *Nannochloropsis oceanica* CCMP1779. *PLoS Genetics*, 8(11). <https://doi.org/10.1371/journal.pgen.1003064>
- Vogelstein, B., & Kinzler, K. W. (1999). Digital PCR. *Proceedings of the National Academy of Sciences of the United States of America*, 96(16), 9236–9241. <https://doi.org/10.1073/pnas.96.16.9236>
- Vu, C. H. T., Lee, H. G., Chang, Y. K., & Oh, H. M. (2018). Axenic cultures for microalgal biotechnology: Establishment, assessment, maintenance, and applications. *Biotechnology Advances*, 36(2). <https://doi.org/10.1016/j.biotechadv.2017.12.018>
- Wan P., Tao, M., Zhou, Y., Han, W., Wang, J., Wang, J. (2024). Cryo-SEM investigation of *Chlorella* using filter paper as substrate. *Bio-Protocol*, 4(24):e5143. <https://doi.org/10.21769/BioProtoc.5143>
- Wang, Y., Hammes, F., De Roy, K., Verstraete, W., & Boon, N. (2010). Past, present and future applications of flow cytometry in aquatic microbiology. *Trends in Biotechnology*, 28(8), 416-424. <https://doi.org/10.1016/j.tibtech.2010.04.006>
- Wang, H., Laughinghouse, H. D., Anderson, M. A., Chen, F., Williams, E., Place, A. R., Zmora, O., Zohar, Y., Zheng, T., & Hill, R. T. (2012). Novel bacterial isolate from permian

- groundwater, capable of aggregating potential biofuel-producing microalga *Nannochloropsis oceanica* IMET1. *Applied and Environmental Microbiology*, 78(5), 1445 - 1453. <https://doi.org/10.1128/AEM.06474-11>
- Wang, D., Ning, K., Li, J., Hu, J., Han, D., Wang, H., Zeng, X., Jing, X., Zhou, Q., Su, X., Chang, X., Wang, A., Wang, W., Jia, J., Wei, L., Xin, Y., Qiao, Y., Huang, R., Chen, J., ... Xu, J. (2014). *Nannochloropsis* genomes reveal evolution of microalgal oleaginous traits. *PLoS Genetics*, 10(1). <https://doi.org/10.1371/journal.pgen.1004094>
- Wang, R., Xue, S., Zhang, D., Zhang, Q., Wen, S., Kong, D., Yan, C., & Cong, W. (2015). Construction and characteristics of artificial consortia of *Scenedesmus obliquus*-bacteria for *S. obliquus* growth and lipid production. *Algal Research*, 12, 436-445. <https://doi.org/10.1016/j.algal.2015.10.002>
- Wang, H., Hill, R. T., Zheng, T., Hu, X., & Wang, B. (2016). Effects of bacterial communities on biofuel-producing microalgae: Stimulation, inhibition and harvesting. *Critical Reviews in Biotechnology*, 36(2), 341-352. <https://doi.org/10.3109/07388551.2014.961402>
- Wang, B., & Jia, J. (2020). Photoprotection mechanisms of *Nannochloropsis oceanica* in response to light stress. *Algal Research*, 46, 380-396. <https://doi.org/10.1016/j.algal.2019.101784>
- Wang, X. J., Zhang, N., Teng, Z. J., Wang, P., Zhang, W. P., Chen, X. L., Zhang, Y. Z., Chen, Y., Fu, H. H., & Li, C. Y. (2021). Structural and mechanistic insights into dimethylsulfoxide formation through dimethylsulfide oxidation. *Frontiers in Microbiology*, 12. <https://doi.org/10.3389/fmicb.2021.735793>
- Wang, Y., Chen, P., Yu, X., & Zhang, J. (2022). Algae-bacteria symbiotic constructed wetlands for antibiotic wastewater purification and biological response. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.1044009>
- Wang, Y., Ning, W., Li, S., Gao, C., Cui, R., Guo, W., Chang, J. S., & Ho, S. H. (2023a). Metabonomics analysis of microalga *Scenedesmus obliquus* under ciprofloxacin stress. *Environmental Research*, 237. <https://doi.org/10.1016/j.envres.2023.116974>
- Wang, Y., Gallagher, L. A., Andrade, P. A., Liu, A., Humphreys, I. R., Turkarslan, S., Cutler, K. J., Arrieta-Ortiz, M. L., Li, Y., Radey, M. C., McLean, J. S., Cong, Q., Baker, D., Baliga, N. S., Peterson, S. B., & Mougous, J. D. (2023b). Genetic manipulation of *Patescibacteria* provides mechanistic insights into microbial dark matter and the epibiotic lifestyle. *Cell*, 186(22). <https://doi.org/10.1016/j.cell.2023.08.017>
- Wang, H., Zheng, L., Tan, C., Li, L., Liu, F., & Feng, H. (2024). Changes in the microbial community structure and diversity during the electricity generation process of a microbial fuel cell with algal-film cathode. *Clean Energy*, 8(5), 177-185. <https://doi.org/10.1093/ce/zkae066>

- Wei, J., Gao, L., Shen, G., Yang, X., & Li, M. (2020). The role of adsorption in microalgae biological desalination: Salt removal from brackish water using *Scenedesmus obliquus*. *Desalination*, 493. <https://doi.org/10.1016/j.desal.2020.114616>
- Wei, X., Shi, F., Chen, Z., Feng, J., & Zhu, L. (2023). Response of bacterial communities (Marivita, Marinobacter, and Oceanicaulis) in the phycosphere to the growth of *Phaeodactylum tricornutum* in different inorganic nitrogen sources. *Frontiers in Marine Science*, 10. <https://doi.org/10.3389/fmars.2023.1086166>
- Westerhoff, P., Hu, Q., Esparza-Soto, M., & Vermaas, W. (2010). Growth parameters of microalgae tolerant to high levels of carbon dioxide in batch and continuous-flow photobioreactors. *Environmental Technology*, 31(5). <https://doi.org/10.1080/09593330903552078>
- Whale, A. S., De Spiegelaere, W., Trypsteen, W., Nour, A. A., Bae, Y. K., Benes, V., Burke, D., Cleveland, M., Corbisier, P., Devonshire, A. S., Dong, L., Drandi, D., Foy, C. A., Garson, J. A., He, H. J., Hellemans, J., Kubista, M., Lievens, A., Makrigiorgos, M. G., ... Huggett, J. F. (2020). The Digital MIQE Guidelines Update: Minimum Information for Publication of Quantitative Digital PCR Experiments for 2020. *Clinical Chemistry*, 66(8), 1012–1029. <https://doi.org/10.1093/clinchem/hvaa125>
- Whitman, W. B., Oren, A., Chuvochina, M., da Costa, M. S., Garrity, G. M., Rainey, F. A., Rossello-Mora, R., Schink, B., Sutcliffe, I., Trujillo, M. E., & Ventura, S. (2018). Proposal of the suffix –ota to denote phyla. Addendum to ‘proposal to include the rank of phylum in the international code of nomenclature of prokaryotes.’ *International Journal of Systematic and Evolutionary Microbiology*, 68(3). <https://doi.org/10.1099/ijsem.0.002593>
- Whitman, W. B., Chuvochina, M., Hedlund, B. P., Hugenholtz, P., Konstantinidis, K. T., Murray, A. E., Palmer, M., Parks, D. H., Probst, A. J., Reysenbach, A. L., Rodriguez-R, L. M., Rossello-Mora, R., Sutcliffe, I., & Venter, S. N. (2022). Development of the SeqCode: A proposed nomenclatural code for uncultivated prokaryotes with DNA sequences as type. *Systematic and Applied Microbiology*, 45(4). <https://doi.org/10.1016/j.syapm.2022.126305>
- Wienhausen, G., Dlugosch, L., Jarling, R., Wilkes, H., Giebel, H. A., & Simon, M. (2022). Availability of vitamin B12 and its lower ligand intermediate α -ribazole impact prokaryotic and protist communities in oceanic systems. *ISME Journal*, 16(8), 2002–2014. <https://doi.org/10.1038/s41396-022-01250-7>
- Wu, Y. W., Simmons, B. A., & Singer, S. W. (2016). MaxBin 2.0: An automated binning algorithm to recover genomes from multiple metagenomic datasets. *Bioinformatics*, 32(4), 605–607. <https://doi.org/10.1093/bioinformatics/btv638>
- Wynne, M. J., & Hallan, J. K. (2016). Reinstatement of *Tetradismus* G. M. Smith (Sphaeropleales, Chlorophyta). *Feddes Repertorium*, 126(3–4), 83–86. <https://doi.org/10.1002/fedr.201500021>

- Xiao, R., & Zheng, Y. (2016). Overview of microalgal extracellular polymeric substances (EPS) and their applications. *Biotechnology Advances*, 34(7), 1225-1244.
<https://doi.org/10.1016/j.biotechadv.2016.08.004>
- Xiao, Y., Zhang, J., Cui, J., Feng, Y., & Cui, Q. (2013). Metabolic profiles of *Nannochloropsis oceanica* IMET1 under nitrogen-deficiency stress. *Bioresource Technology*, 130, 731-738.
<https://doi.org/10.1016/j.biortech.2012.11.116>
- Yadav, G., Dash, S. K., & Sen, R. (2019). A biorefinery for valorization of industrial wastewater and flue gas by microalgae for waste mitigation, carbon-dioxide sequestration and algal biomass production. *Science of the Total Environment*, 688, 129-135.
<https://doi.org/10.1016/j.scitotenv.2019.06.024>
- Yakimov, M. M., Merkel, A. Y., Gaisin, V. A., Pilhofer, M., Messina, E., Hallsworth, J. E., Klyukina, A. A., Tikhonova, E. N., & Gorlenko, V. M. (2022). Cultivation of a vampire: ‘*Candidatus Absconditicoccus praedator*.’ *Environmental Microbiology*, 24(1), 30-49.
<https://doi.org/10.1111/1462-2920.15823>
- Yang, Y., & Gao, K. (2003). Effects of CO₂ concentrations on the freshwater microalgae, *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa* and *Scenedesmus obliquus* (Chlorophyta). *Journal of Applied Phycology*, 15, 379–389.
<https://doi.org/10.1023/A:1026021021774>
- Yao, S., Lyu, S., An, Y., Lu, J., Gjermansen, C., & Schramm, A. (2019). Microalgae–bacteria symbiosis in microalgal growth and biofuel production: a review. *Journal of Applied Microbiology*, 126(2), 359–368. <https://doi.org/10.1111/jam.14095>
- Yen, H. W., Ho, S. H., Chen, C. Y., & Chang, J. S. (2015). CO₂, NO_x and SO_x removal from flue gas via microalgae cultivation: A critical review. *Biotechnology Journal*, 10(6), 829-839. <https://doi.org/10.1002/biot.201400707>
- Yoo, C., Jun, S. Y., Lee, J. Y., Ahn, C. Y., & Oh, H. M. (2010). Selection of microalgae for lipid production under high levels carbon dioxide. *Bioresource Technology*, 101, S71-S74.
<https://doi.org/10.1016/j.biortech.2009.03.030>
- Yoon, H. S., Hackett, J. D., Ciniglia, C., Pinto, G., & Bhattacharya, D. (2004). A Molecular timeline for the origin of photosynthetic eukaryotes. *Molecular Biology and Evolution*, 21(5), 809–818. <https://doi.org/10.1093/molbev/msh075>
- Yoshihara, K. I., Nagase, H., Eguchi, K., Hirata, K., & Miyamoto, K. (1996). Biological elimination of nitric oxide and carbon dioxide from flue gas by marine microalga NOA-113 cultivated in a long tubular photobioreactor. *Journal of Fermentation and Bioengineering*, 82(4), 351-354. [https://doi.org/10.1016/0922-338X\(96\)89149-5](https://doi.org/10.1016/0922-338X(96)89149-5)
- Yoshinari, A., Isoda, R., Yagi, N., Sato, Y., Lindeboom, J. J., Ehrhardt, D. W., Frommer, W. B., & Nakamura, M. (2024). Near-infrared imaging of phytochrome-derived autofluorescence

- in plant nuclei. *Plant Journal*, 118(5), 1699–1712. <https://doi.org/10.1111/tpj.16699>
- Yu, H., Kim, J., Rhee, C., Shin, J., Shin, S. G., & Lee, C. (2022). Effects of different pH control strategies on microalgae cultivation and nutrient removal from anaerobic digestion effluent. *Microorganisms*, 10(2). <https://doi.org/10.3390/microorganisms10020357>
- Yu, H., Ko, D., & Lee, C. (2023). Continuous cultivation of mixed-culture microalgae using anaerobic digestion effluent in photobioreactors with different strategies for adjusting nitrogen loading rate. *Bioresource Technology*, 387. <https://doi.org/10.1016/j.biortech.2023.129650>
- Yu, Q., Pei, X., Wei, Y., Naveed, S., Wang, S., Chang, M., Zhang, C., & Ge, Y. (2023). The roles of bacteria in resource recovery, wastewater treatment and carbon fixation by microalgae-bacteria consortia: A critical review. *Algal Research*, 69. <https://doi.org/10.1016/j.algal.2022.102938>
- Yu, Y., Lee, C., Kim, J., & Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnology and Bioengineering*, 89(6), 670-679. <https://doi.org/10.1002/bit.20347>
- Yuan, T., Xue, Y., Wang, C., Gao, P., Zhao, B., Zhao, L., Jin, C., Li, S., & He, B. (2025). Calculation of CO₂ emissions from coal-fired power plants based on OCO-2/3 satellite observations and divergence model. *Energy*, 315, 134303. <https://doi.org/10.1016/j.energy.2024.134303>
- Zachar, I., & Boza, G. (2020). Endosymbiosis before eukaryotes: mitochondrial establishment in protoeukaryotes. *Cellular and Molecular Life Sciences*, 77(18), 3503–3523. <https://doi.org/10.1007/s00018-020-03462-6>
- Zaytsev, P. A., Rodin, V. A., Zaytseva, A. A., Zvereva, M. I., & Solovchenko, A. E. (2024). Advances of high-throughput sequencing for unraveling biotechnological potential of microalgal-bacterial communities. *Journal of Applied Phycology*, 36(4), 1901–1919. <https://doi.org/10.1007/s10811-024-03267-1>
- Zengler, K., & Zaramela, L. S. (2018). The social network of microorganisms - How auxotrophies shape complex communities. *Nature Reviews Microbiology*, 16(6), 383–390. <https://doi.org/10.1038/s41579-018-0004-5>
- Zhang, B., Lens, P. N. L., Shi, W., Zhang, R., Zhang, Z., Guo, Y., Bao, X., & Cui, F. (2018). Enhancement of aerobic granulation and nutrient removal by an algal–bacterial consortium in a lab-scale photobioreactor. *Chemical Engineering Journal*, 334, 2373-2382. <https://doi.org/10.1016/j.cej.2017.11.151>
- Zhang, B., Chen, J., Su, Y., Sun, W., & Zhang, A. (2021). Utilization of indole-3-acetic acid–secreting bacteria in algal environment to increase biomass accumulation of *Ochromonas* and *Chlorella*. *Bioenergy Research*, 15(1), 242–252. <https://doi.org/10.1007/s12155-021->

- Zhang, J., Liu, R., Xi, S., Cai, R., Zhang, X., & Sun, C. (2020). A novel bacterial thiosulfate oxidation pathway provides a new clue about the formation of zero-valent sulfur in deep sea. *ISME Journal*, 14(9), 2261–2274. <https://doi.org/10.1038/s41396-020-0684-5>
- Zhang, M., Wang, H., & Chen, F. (2022). Time-resolved transcriptome analysis of *Scenedesmus obliquus* HTB1 under 10% CO₂ condition. *Microbial Biotechnology*, 16(2), 448-462. <https://doi.org/10.1111/1751-7915.14100>
- Zhang, Y., Zheng, L., Wang, S., Zhao, Y., Xu, X., Han, B., & Hu, T. (2022). Quorum sensing bacteria in the phycosphere of HAB microalgae and their ecological functions related to cross-kingdom interactions. *International Journal of Environmental Research and Public Health*, 19(1). <https://doi.org/10.3390/ijerph19010163>
- Zhang, Y., Ren, L., Chu, H., Zhou, X., Yao, T., & Zhang, Y. (2019). Optimization for *Scenedesmus obliquus* cultivation: The effects of temperature, light intensity and pH on growth and biochemical composition. *Microbiology and Biotechnology Letters*, 47(4), 614-620. <https://doi.org/10.4014/mbl.1906.06005>
- Zhilina, T. N., Sorokin, D. Y., Toshchakov, S. V., Kublanov, I. V., & Zavarzina, D. G. (2023). *Natronogracilivirga saccharolytica* gen. nov., sp. nov. and *Cyclonatronum proteinivorum* gen. nov., sp. nov., haloalkaliphilic organotrophic Bacteroidetes from hypersaline soda lakes forming a new family Cyclonatronaceae fam. nov. in the order Balneolales. *Systematic and Applied Microbiology*, 46(2). <https://doi.org/10.1016/j.syapm.2023.126403>
- Zhou, Z., Tran, P. Q., Breister, A. M., Liu, Y., Kieft, K., Cowley, E. S., Karaoz, U., & Anantharaman, K. (2022). METABOLIC: high-throughput profiling of microbial genomes for functional traits, metabolism, biogeochemistry, and community-scale functional networks. *Microbiome*, 10(1). <https://doi.org/10.1186/s40168-021-01213-8>
- Zhu, J., Yu, Z., He, L., Cao, X., Wang, W., & Song, X. (2024). Phycosphere bacterial composition and function in colony and solitary *Phaeocystis globosa* strains providing novel insights into the algal blooms. *Marine Pollution Bulletin*, 206, 116700. <https://doi.org/10.1016/j.marpolbul.2024.116700>