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Unraveling the Metabolic Potential of Pelagic *Sargassum*-Associated Microbes: Insights into siderophore production, biosynthesis, and phosphonate degradation

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology, Evolution, and Marine Biology

by

Taruna Aggarwal Schuelke

Committee in charge:

Professor Elizabeth G. Wilbanks Chair

Professor Craig Carlson

Professor Alyson Santoro

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The dissertation of Taruna Aggarwal Schuelke is approved.

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Craig Carlson

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Alyson Santoro

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Elizabeth G. Wilbanks, Committee Chair

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Taruna A. Schuelke
Ecology, Evolution, and Marine Biology | University of California, Santa Barbara
taruna@ucsb.edu

EDUCATION

University of California, Santa Barbara
Ph.D. – Ecology, Evolution, and Marine Biology, 2024

University of New Hampshire
M.Sc. – Genetics, 2016

University of California, Davis
B.Sc. – Genetics, 2010

PUBLICATIONS

- Sackett JD, Kamble N, Leach E. **Schuelke TA**, Wilbanks E, Rowe AR. 2022. Genome-Scale Mutational Analysis of Cathode-Oxidizing *Thioclava electrotropha* Elox9T. *Frontiers in Microbiology*: 13:909824.
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- UC President's Dissertation Year Fellowship ($32,499), UC Santa Barbara, 2022
- Graduate Research Mentorship Fellowship ($24,000), UC Santa Barbara, 2022
- Xsede Access ($349), 2000 Service Units for HPCC, National Science Foundation, 2022
- Graduate Opportunity Fellowship ($24,000), UC Santa Barbara, 2021
- Schmidt Family Fountain Research Accelerator Award ($8,000), UC Santa Barbara, 2020
- Excellence in Teaching Award Nominee, UC Santa Barbara, 2020
- New Hampshire Agricultural Experiment Station, Research Award ($5,546), University of New Hampshire, 2016

ABSTRACT

Unraveling the Metabolic Potential of Pelagic *Sargassum*-Associated Microbes: Insights into siderophore production, biosynthesis, and phosphonate degradation

by

Taruna Aggarwal Schuelke

Macroalgae attract a diverse array of microorganisms to their surfaces by exuding nutrient-rich organic matter, which leads to microbial colonization and biofilm formation. These microbes play a variety of crucial roles in maintaining the overall health of the ecosystem. To fully understand these beneficial interactions and uncover additional, less apparent microbial functions, it is imperative to study the genetic composition of these microorganisms and explore their metabolic potential.

My research delves into these metabolic capabilities by studying the microbes that live on, in, and around a photosynthetic macroalga known as *Sargassum*. While many *Sargassum* species are benthic, two species, *S. natans* and *S. fluitans*, are holopelagic, meaning they float freely in the open ocean without attaching to any substrates. These species are typically found in the nutrient-poor subtropical waters of the North Atlantic Ocean, especially the Sargasso Sea. Since 2011, however, *Sargassum's* range and abundance have expanded significantly beyond the subtropical North Atlantic into the tropical Atlantic

waters. Various environmental factors have contributed to the recurring blooms of *Sargassum*, which can span from West Africa to the Gulf of Mexico.

By examining the genetic profiles of the microbes associated with *Sargassum*, I aim to understand their roles in (1) iron acquisition via siderophores and (2) the remineralization of phosphonates. Siderophores are iron-chelating molecules and phosphonates are an important phosphorus (P) source in P-limited systems such as the Sargasso Sea. Both of these nutrients are essential for *Sargassum* to perform photosynthesis. Using amplicon and metagenomic data, we identified complete and partial gene clusters that *Sargassum*'s microbial symbionts leveraged to biosynthesize siderophores like amphibactins, ferrioxamines, and ferrichromes. We also detected genes involved in several phosphonate degradation pathways in free-living bacteria found inside and outside *Sargassum* communities of Sargasso Sea. By revealing these genetic capabilities, our research seeks to add another piece to the puzzle that explains the success and persistence of *Sargassum* in the oligotrophic conditions of the Sargasso Sea and beyond.

Table of Contents

Chapter 1. Community Structure of Pelagic *Sargassum's* Microbiome

Taruna A. Schuelke, Martha Gledhill, Elizabeth Wilbanks

1.1 Introduction

1.1.1 The pelagic *Sargassum*

Sargassum, a type of brown macroalga, is common seaweed found in tropical and subtropical waters across the globe. While most species within the *Sargassum* genus are benthic, two holopelagic species, *S. natans* and *S. fluitans*, are the defining feature of the Sargasso Sea in western north Atlantic Ocean. These *Sargassum* species form expansive floating mats on the sea surface that provide a safe harbor and foraging grounds to more than 100 types of fish, four species of sea turtles, and 145 invertebrates (Chávez et al., 2020; Mansfield et al., 2014; Thiel & Gutow, 2005). *Sargassum* is also known to produce and release exudates that are rich in organic matter (Powers et al., 2019), which supports the colonization of diverse microorganisms on its surface. Pelagic *Sargassum* is a crucial ecosystem for maintaining the biodiversity in the Atlantic Ocean.

1.1.2 The *Sargassum* bloom

Since 2011, there has been a significant increase in both the abundance and geographic range of pelagic *Sargassum*, making this bloom a pronounced feature in the tropical Atlantic Ocean (M. Wang et al., 2019). The geographic region where the *Sargassum* bloom persists year to year have been dubbed the Great Atlantic Sargassum Belt (GASB) shown in Figure 1.1, along with our sampling site (M. Wang et al., 2019). In 2022, the *Sargassum* bloom set a new historical record reaching a biomass of more than 24 million tons (Hu & Barnes, 2022). The causes behind the surge in *Sargassum* abundance are not well understood. Nevertheless, it has been proposed that higher nitrogen introduction from the Amazon plume and/or

increased upwelling off West Africa may explain the development of the bloom in the tropical Atlantic Ocean (Johns et al., 2020; Oviatt et al., 2019; M. Wang et al., 2019).

The higher abundance of *Sargassum* has led to more frequent beaching events in the Gulf of Mexico and the Caribbean, which have serious environmental, ecological, and economic consequences. For example, the Mexican Caribbean coastline experienced a vast beaching event of pelagic *Sargassum* in 2018. Consequently, the combined effect of low oxygen conditions, high ammonium, and high hydrogen sulfide concentrations resulted in nearly 80 faunal species dying (Rodríguez-Martínez et al., 2019). Hydrogen sulfide is also highly toxic to human health (Doujaiji & Al-Tawfiq, 2010; Rodríguez-Martínez et al., 2019). Among the marine animals that are negatively affected by the *Sargassum* bloom and beaching are endangered species of nesting sea turtles (e.g., loggerhead and hawksbill sea turtles). These species rely on sandy beaches to lay their eggs (Maurer et al., 2015), and large seaweed beachings obstruct the success of this key process in their life cycle.

The *Sargassum* bloom is also altering the composition of many native coastal communities. For example, van Tussenbroek et al., (2017) reported that decomposition of drifting *Sargassum* increases the turbidity of the water and reduces light, oxygen, and pH. These murky shore waters caused by decaying algal masses resulted in the loss of *Thalassia testudinum* seagrass meadows and coral reefs in the Caribbean. The replacement and displacement of corals by macroalgae, known as the coral-algal phase shift, are becoming increasingly common (Done, 1992) and increased microbialization of reef systems (Haas et al., 2016; Kelly et al., 2022). Macroalgal species like *Sargassum* have annual life cycles, whereas coral reefs take hundreds to thousands of years to form, and these phase-shifts can

result in reduced ecosystem maturity and lower overall biodiversity (Ainsworth & Mumby, 2015; Done, 1992; Weatherdon et al., 2016).

The negative effects of *Sargassum* beaching events are not only ecological but also economic. In 2018, a peak bloom year, clean-up and restoration efforts cost an estimated 120 million USD in the Caribbean (Rodríguez-Martínez et al., 2023). Mexico has spent some 17 million USD in clean-up costs since the start of the bloom and even deployed its navy researchers to assist in finding other clearing and restoration methods. The Caribbean nations have also experienced reduced tourism, an industry that accounted for 14% of the total economy in 2019. Thus, it is an urgent requirement to understand the drivers behind *Sargassum* bloom.

1.1.3 The microbiomes of macroalgae and their contribution to host health

The intricate symbiotic relationships among the macrofauna, microorganisms, and their *Sargassum* hosts form a complex ecosystem. For example, Lapointe and colleagues (2014) posit that ammonium and soluble reaction phosphorus in fish excretions could serve as important nutrient sources for supporting *Sargassum* growth and biomass (Lapointe et al., 2014). Similar to the potential niche that macrofauna occupy, microorganisms remineralize organic compounds, fix nitrogen, respire carbon dioxide, which are all provisions that macroalga need to perform photosynthesis (Goecke et al., 2010).

The relationship between macroalgae and the microorganisms that form biofilms on their surfaces is often mutually beneficial. One of the earliest studies that examined how bacterial symbionts influence the growth and morphogenesis of macroalgae utilized *Ulva* as a model system. Provasoli and Pintner (1980) revealed that some strains of *Ulva* lost their ability to develop normally in the absence of certain microflora. However, these axenic *Ulva*

strains regained their normal morphology when grown with collections of specific bacterial strains (Provasoli & Pintner, 1980). Furthermore, Matsuo et al., (2003, 2005) were the first to identify thallusin as an algal morphogenesis inducer produced by bacterial isolates from the Cytophaga-Flavobacterium-Bacteroides. In addition to algal growth- and morphogenesis-promoting factors (AGMPFs) produced by their microbial epibionts, macroalgae depend on these associates for a supply of limiting nutrients such as nitrogen and phosphorus. For instance, several investigations have shown that epiphytic microbes may provide fixed nitrogen for macroalgae (Carpenter, 1972; Carpenter & Cox, 1974; Dromgoole et al., 1978; Head & Carpenter, 1975; Raut et al., 2018).

It is evident from previous research that microbial communities are ubiquitous across macroalgal groups. However, laboratory settings limit the number of organisms that can be cultured, which in turn hampers the number of organisms that can be investigated. Today, advances in sequencing technologies have enabled researchers to explore the biodiversity of macroalgal microbiomes on a larger scale. Furthermore, we can examine the genetic makeup and the metabolic abilities of hundreds of microbes simultaneously. This study focuses on uncovering the microbial community composition of pelagic *Sargassum* (this chapter), and their role in nutrient acquisition (Chapters 2 & 3).

1.1.4 Current insights into the microbiome of Pelagic *Sargassum*

Only a handful of investigations characterizing the microbial communities associated with pelagic *Sargassum* have been published (Hervé et al., 2021; Michotey et al., 2020; Theirlynck et al., 2023; Torralba et al., 2017). In 2017, Torralba and colleagues investigated the impact of the Deepwater Horizon oil spill on the microbial communities of pelagic *Sargassum* and its surrounding waters in the Gulf of Mexico. They sequenced the V1-V3

variable region of 16S rRNA from non-oiled and oiled *Sargassum* samples collected in 2010. They discovered that the microbial communities of pelagic *Sargassum* comprised mostly of phyla *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Verrucomicrobia*, with *Rhodobacteraceae* dominating the overall community. Members of these phyla were also present in the pelagic *Sargassum* samples collected from the Great Atlantic Sargassum Belt by Michotey et al., (2020) and Theirlynck et al., (2023). For instance, using 16S rRNA data (V4 region), the two teams of investigators found *Pseudomonadaceae, Alteromonadaceae*, *Rhodobacteraceae*, and *Vibrionaceae* were common families associated with their pelagic *Sargassum* samples. These studies show that many microorganisms associated with pelagic *Sargassum* belong to taxonomic groups known for photoheterotrophy and degradation of complex carbon compounds.

Although the above studies have contributed greatly to our knowledge of *Sargassum* microbiome, we know little about the metabolic capabilities of these microorganisms. Our current research aims to fill that knowledge gap and to explore the nutritional role of the microbiome associated with pelagic *Sargassum* in the North Atlantic Ocean. Our study marks one of the first functional investigations of microbes linked to this ecologically significant macroalga. We hope our work will provide more insights into how *Sargassum's* abundance and geographic range have increased over thousands of miles of pelagic habitat in a short time frame. We describe the microbial community composition associated with pelagic *Sargassum* samples, that were collected aboard a GEOTRACES process cruise in 2018, the year which experienced a large *Sargassum* bloom. We also compare and contrast our findings to those of Theirlynck et al., (2023) which was conducted in the approximate vicinity of the present study.

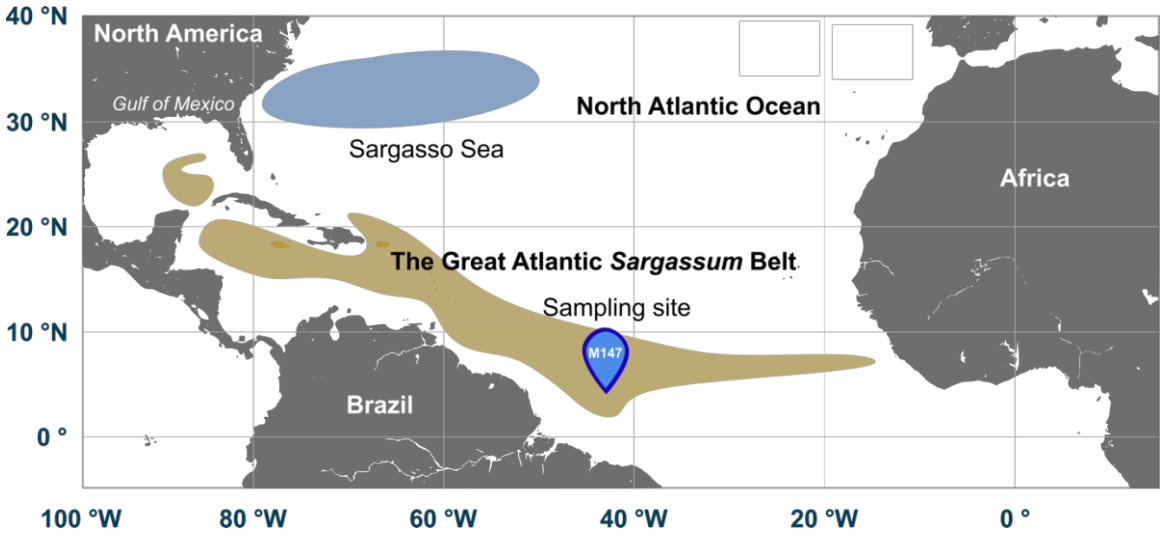


Figure 1.1. Map depicting the Great Atlantic *Sargassum* Belt along with our sampling site. Regenerated using satellite-based images from Wang et al., (2019).

1.2 Methods

1.2.1 Sampling *Sargassum*

Six *Sargassum* samples were collected from the sea surface aboard the R/V *Meteor* on April 27, 2018, during the M147 GEOTRACES process cruise (GApr11; 4°9'0" N 42°53'60" W). Three of these samples were frozen at -80°C immediately upon collection to be used as our controls. These tissue samples are designated as CA, CB, and CC where C stands for control and A-C are biological replicates. The other three tissue samples were used for onboard incubations with the goal of assessing (a) which microbes respond to the dissolved organic carbon that is exuded from *Sargassum* and (b) which metabolites, namely siderophores, are these microorganisms capable of biosynthesizing and secreting. The experimentally incubated samples are EA, EB, and EC where E stands for experimental and A-C are biological replicates. We describe our experimental setup in this chapter (Figure 1.2), and we present our nutrient concentrations and tissue content results in this chapter along with an overview of the metabolic capabilities of these microorganisms. We reserve the detailed

description of the siderophores produced by these microorganisms and their biosynthetic genetic abilities for Chapter 2.

1.2.2 Incubation experiments for *Sargassum*

Seawater from 500 m depth at the *Sargassum* collection site was 0.22-µm filtered and used for incubating the *Sargassum* tissue. We used nutrient-rich, deep-sea water to alleviate macronutrient limitation. Six trace-metal clean 2L polycarbonate bottles were filled with 2L of the 0.22-µm filtered deep seawater. *Sargassum* tissue was placed in three experimental bottles (i.e., EAw, EBw, ECw), and the other three bottles were designated negative controls containing no tissue (i.e., CAw, CBw, CCw). All six bottles were incubated in a deckboard incubator for 24 hours. Here, C and E in the naming scheme indicate control and experimental samples and w stands for water. Letters A-C denote biological replicates.

Tissue samples were collected before and after the incubation period for elemental analysis and molecular work. Seawater samples were collected before and after the incubation period for measuring macronutrients. The incubated water samples from all six bottles were also filtered using a 0.22-µm polyvinylidene fluoride membrane filter (Sterivex-GV, Millipore) and then passed through a 200-mg modified polystyrene divinyl benzene SPE column cartridge (ENV+, Biotage, Sweden) at ambient pH. Extractions were carried out at room temperature in the dark. A vacuum manifold was used at a flow rate of ~10 mL min^{-1} during filtration and solid phase extraction processes (Gledhill, Hollister, et al., 2022). The Sterivex-GV and column cartridges were dried and frozen at -20°C for future processing at GEOMAR. SPE samples were also taken to measure dissolved organic carbon (DOC). The Sterivex-GV filters and the ENV+ columns were used to detect particulate and dissolved metabolites, respectively. An overview of the experimental setup is presented in Figure 1.2.

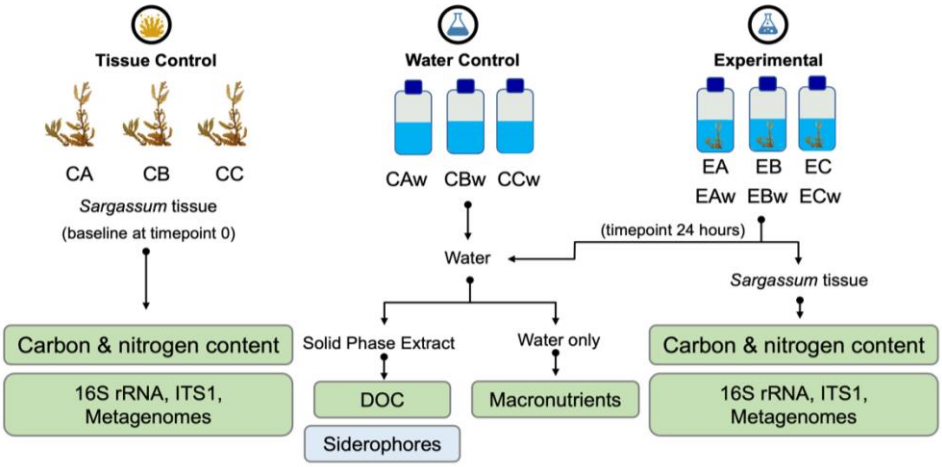


Figure 1.2. An overview of the experimental design implemented during the M147 GEOTRACES process cruise in April 2018. The data types in green and blue boxes are discussed in this chapter and chapter 2, respectively.

1.2.3 Seawater nutrient analysis

Dissolved macronutrient concentrations (phosphate, nitrate, and nitrite) were determined on board using a continuous flow auto analyzer (SEAL QuAAtro). Reference materials (Kanso Co, Japan) were analyzed alongside samples in each run as quality control.

1.2.4 Carbon and nitrogen content of *Sargassum*

Carbon and nitrogen contents of dried (50°C, 24 hours) *Sargassum* tissue were determined with an elemental analyzer (Euro Elemental Analyzer), using acetanilide as standard.

1.2.5 DNA extraction and library preparation

Sargassum tissue samples were stored at -80°C and were kept on dry ice during processing. Sterile techniques were used throughout sample preparation and DNA extraction assays. To prepare *Sargassum* samples for DNA extraction, tissues were ground in liquid nitrogen and immediately transferred to 15 mL falcon tubes on dry ice until the next step. We did not weigh the samples to avoid thawing the tissue, but we did add approximately the same amount of tissue to each falcon tube.

DNA from the six *Sargassum* samples was extracted using a modified version of the cetyltrimethylammonium bromide (CTAB) protocol (Appendix § I) (Arseneau et al., 2017; Ausubel et al., 2003). Briefly, we added lysis buffer and lysozyme to lyse the cells and incubated these samples at 37°C for one hour with periodic mixing. Next, the CTAB extraction buffer and Proteinase-K were added to the samples, followed by incubation at 55°C for 3 hours. Afterwards, the samples were cooled to room temperature and processed with chloroform:isoamyl alcohol (24:1) to separate the organic phase from the aqueous phase. The aqueous phase was removed to clean tubes and the DNA was precipitated with CTAB precipitation buffer, followed by two 80% ethanol washes. The cleaned DNA was resuspended in 50 µl nuclease-free water.

The DNA concentrations were quantified on the Qubit Fluorometer 4.0, and the purity ratios were measured on the NanoDrop Spectrophotometer 1000. DNA fragment sizes were assessed on Agilent TapeStation Analyzer. Samples with concentrations ≥ 3 ng μL^{-1}, with optimal purity ratios, and with high molecular weight DNA were treated with RNase I_f (NEB, cat. no. M0243S) following the manufacturer's recommendation. The RNase I_f was inactivated by incubating the samples at 70°C for 15 minutes. Some of the resulting extracts were cleaned with AMPureXP beads (Beckman Coulter, cat. no. A63881) to remove the RNase I_f enzyme, while others were not purified in accordance with UC Davis Genome Center's guidelines to avoid losing DNA. It is worth pointing out that the RNase treatment step should be implemented during the DNA extraction assay rather than at the end of the protocol. We encountered various issues while performing post-extraction RNase treatments such as loss of valuable DNA.

16S SSU rRNA (V4 region) and ITS1 (ITS1 region) amplicon libraries were generated following the Earth Microbiome Project protocol (Thompson et al., 2017). The forward and reverse 16S SSU rRNA primers were 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) (Apprill et al., 2015; Parada et al., 2016). The forward and reverse ITS1 primers were ITS1f (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) (Hoggard et al., 2018). 50 μL PCR reactions contained 13 μL Q5 High-Fidelity 2X master mix (NEB, cat. no. M0492S), 1 μL of forward and reverse primer mixture, nuclease-free water, and DNA depending on the concentration. We added at least 1 ng μL^{-1} of DNA to each PCR reaction. PCR-grade water and the ZymoBIOMICS microbial community DNA standard (Zymo Research, cat. no. D6305) were used as negative and positive PCR controls, respectively. The PCR conditions for 16S rRNA and ITS1 genes are listed in Table 1.1. The resulting libraries were purified, size-selected using magnetic beads, and sequenced on the MiSeq platform (Illumina) at the Biological Nanostructures Laboratory, Univ. of Calif., Santa Barbara.

	16S rRNA	**ITS1**
	98°C for 30 sec	98°C for 30 sec
35 cycles	98°C for 20 sec 55°C for 30 sec 72°C for 30 sec	98°C for 20 sec 53°C for 15 sec 72°C for 20 sec
	72°C for 2 min	72°C for 1 min

Table 1.1. PCR conditions for 16S rRNA and ITS1 gene used in this study.

For metagenomic library preparation, DNA extracts were sheared using the Covaris M220. For each sample, 50 μL of the extract was used to shear the DNA in the microtube-50 AFA fiber screwcap (PN 520166). We followed the Covaris recommended protocol to accomplish the target fragment base pair peak of 200 bp. In general, the fragments tended to be approximately 100 bp larger than the target peak. The DNA fragment size distributions in

each sample were assessed on Agilent TapeStation using the high sensitivity D5000 tape assay (cat. nos. 5067-5592 & 5067-5593). Libraries for sequencing were prepared using the ThruPLEX DNA-Seq HV kit by Takara Bio USA, Inc. (cat. no. R400741) following the company's protocol and were submitted to UC Davis Genome Center for sequencing on the Novaseq 6000 platform (Illumina).

1.2.6 16S rRNA and ITS1 analyses

Demultiplexed 16S rRNA and ITS1 read pairs were imported, visualized, and analyzed in QIIME 2 v2021.2 (Bolyen et al., 2019). The DADA2 package within QIIME 2 was used to resolve biological differences among sequences resulting in amplicon sequence variants (ASVs). For ITS1, only the forward reads were used for clustering because the ITS1 insert size is larger than the read length. Next, the ASVs were taxonomically classified using the SILVA rRNA database v138 (515F-806R region only) and the UNITE database v9 for 16S rRNA and ITS, respectively (released on Nov. 29, 2022). Both databases contained sequences clustered at 99% similarity. Sample by species matrices were constructed in QIIME 2, and the taxonomic identifications for both genes were manually curated. Specifically, we removed all ASVs assigned to eukaryotes, chloroplasts, mitochondria, and unassigned taxa from our 16S samples by species matrix. From the ITS1 matrix, we filtered ASVs of zero abundance. For alpha diversity calculations, we rarefied our data to the smallest count of ASVs.

1.2.7 Metagenomic assembly and annotation

Read quality for each sample was assessed using FastQC (Andrews et al., 2012). We used Trimmomatic v0.39 for read quality control. Adapters and reads were trimmed using default ILLUMINACLIP parameters and using a threshold phred score of 15, respectively (Bolger et

al., 2014). We constructed both single sample assemblies and co-assemblies with MEGAHIT v1.2.9 using all paired and unpaired reads that passed our filtering thresholds (Li et al., 2015). Next, we mapped our trimmed reads from each individual sample to each assembly and co-assembly to obtain differential sequencing coverage information using Bowtie 2 v2.4.4 and SAMtools v1.13.0 (Danecek et al., 2021; Langmead & Salzberg, 2012).

Contigs larger than 2,500 bp, from each assembly type, were binned into metagenome-assembled genomes (MAGs) using MetaBAT2 (D. D. Kang et al., 2019). We used CheckM v1.2.2 to determine assembly and competition statistics (Parks et al., 2015). We conducted taxonomic classification using GTDB-Tk v2.3.0 against the Genome Taxonomy Database R214, and we dereplicated our MAGs with dRep v3.4.3 (Chaumeil et al., 2020; Olm et al., 2017). We identified high- and medium-quality genomes based on the MIMAG standards which stipulate that high-quality MAGs have a completion percentage of >90% and contamination of <5%, while medium-quality MAGs are ≥50% complete with <10% contamination (Bowers et al., 2017). In order to predict metabolic and biogeochemical functional profiles, we used METABOLIC v4.0 (Zhou et al., 2022). We used default parameters for all the tools mentioned in the methods unless otherwise noted.

1.3. Results and discussion

1.3.1 Seawater nutrient concentrations

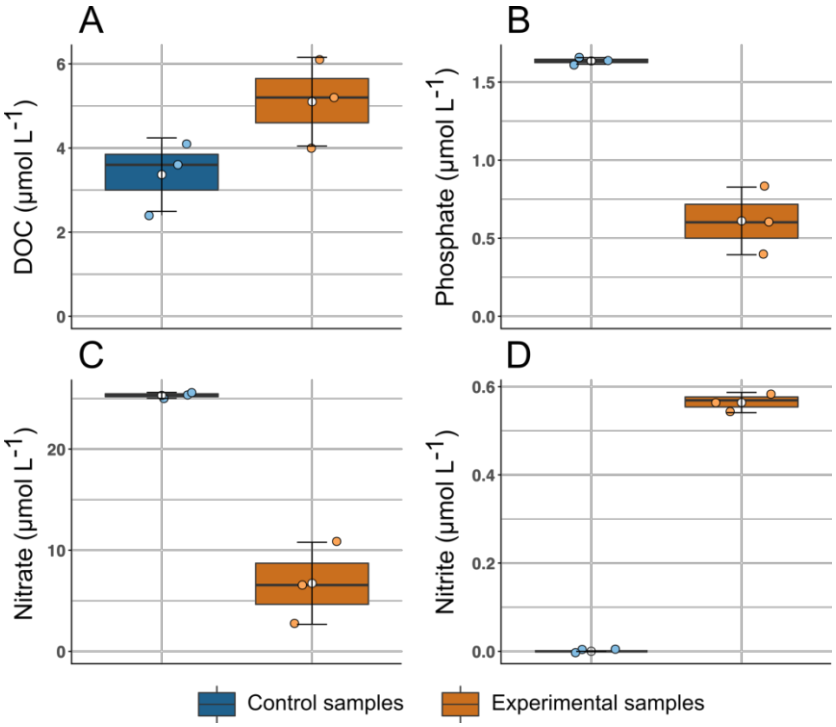


Figure 1.3. Boxplots of triplicate concentrations of dissolved organic carbon (DOC, A), phosphate (B), nitrate (C), and nitrite (D) in the seawater used for the experiment and their corresponding average and standard deviation values. The differences in the three macronutrient types between the control and the experimental samples are statistically significant ($p<0.05$) but the increase in DOC in the incubated samples compared to the control samples was not statistically significant ($p>0.05$). The raw values and their means are represented by the filled and open circles in each boxplot, respectively.

We measured the concentrations of DOC, phosphate, nitrate, and nitrite in the seawater used for incubations in order to track the differences in DOC and these macronutrients between our control and experimental bottles (Figure 1.3). Based on the nonparametric Kruskal-Wallis test, the differences in the three macronutrient types between the control and the experimental samples are statistically significant (Figure 1.3, $p<0.05$), however, the DOC concentrations were not significantly different. We observed an average increase of 2.4 ± 1.9 µmol L^{-1} (+51.5%) in the DOC concentrations in our experimental bottles which contained

Sargassum fronds (Figure 1.3). In contrast, we detected an average drawdown of 1.0 ± 0.2 μmol L^{-1} (-62.6%) and 18.6 ± 3.93 μmol L^{-1} (-73.4%) for phosphate and nitrate concentrations, respectively, in the experimental bottles (Figure 1.3). The increase in DOC levels is consistent with the established understanding that brown macroalgae are significant contributors to the DOC pool (Dolliver & O'Connor, 2022). The consumption of nutrients, especially phosphate, confirms the findings of earlier work conducted by Lapointe and colleagues who found that the growth and photosynthetic rates of phosphate-enriched *Sargassum* cultures were two-fold higher than control cultures (Lapointe, 1986).

We also observed an increase in nitrite concentration from below detection (0.01 μmol L^{-1}) in the control samples at the start of the incubation period to 0.6 ± 0.02 μmol L^{-1} in the final samples at the end of the incubation period (Figure 1.3). The presence of nitrite in our incubations suggests that one or more nitrogen-related processes may be occurring within the microbial consortium associated with *Sargassum*. We discuss these findings in conjunction with our genomic-based results in section 1.3.8.

1.3.2 Carbon to nitrogen content of *Sargassum*

We measured the particulate carbon and nitrogen content of *Sargassum* tissue before starting incubations and after the 24-hour incubation period. The average tissue organic nitrogen content was 0.06 ± 0.015 μmol mg dry wt^{-1} and 0.07 ± 0.004 μmol mg dry wt^{-1} in the control and experimental *Sargassum* tissue, respectively (Table 1.2). The average particulate organic carbon content was 1.98 ± 0.22 μmol mg dry wt^{-1} and 1.88 ± 0.28 μmol mg dry wt^{-1} in the control and experimental *Sargassum* tissue, respectively (Table 1.2). Even though these averages resulted in a lower C:N in the experimental *Sargassum* samples compared to the

control tissues, they were not statistically significant ($p>0.05$) based on the Kruskal-Wallis test.

Sample	Tissue Organic C (μmol mg dry wt^{-1})	Tissue Organic N (μmol mg dry wt^{-1})	C:N	Avg. C:N ± St. dev.
CA	2.20	0.045	48.62	35.08 ± 12.80
CB	1.99	0.059	33.42	
CC	1.76	0.075	23.21	
EA	2.17	0.074	29.23	25.89 ± 2.900
EB	1.83	0.075	24.44	
EC	1.63	0.067	24.00	

Table 1.2. Tissue organic nitrogen (PON), particular organic carbon (POC) contents, and average C:N ratio of control (C) and experimental (E) *Sargassum* tissue samples.

1.3.3 Read filtering and quality control

We obtained an average of 56,149 and 30,984 paired-end reads from the MiSeq runs for 16S rRNA and ITS1, respectively. An average of ~66% and ~69% of the 16S rRNA and ITS1 read pairs passed filtering and were used for clustering into ASVs, respectively (Tables 1.3 and 1.4). A, B, and C represent biological replicates and A.r1-A.r3, B.r1-B.r3, and C.r1-C.r3 are technical replicates.

Sample	Num. read pairs	% read pairs passing filter	% merged read pairs	% non-chimeric read pairs
CA.r1	72,536	77.74	69.22	65.11
CA.r2	69,128	81.2	74.35	70.28
CA.r3	73,710	80.63	73.17	69.76
CB.r1	64,491	83.7	76.53	73.01
CB.r2	69,254	84.98	78.21	73.67
CB.r3	67,584	86.27	79.65	75.26
CC.r1	73,730	88.84	85.77	85.08
CC.r2	60,497	86.75	83.47	82.76
CC.r3	56,797	91.01	88.18	87.48
EA.r1	64,943	81.06	74.37	71.07
EA.r2	64,458	82.5	77.15	72.99
EA.r3	69,098	84.86	80.31	76.48
EB.r1	28,408	81.88	75.38	73.91

EB.r2	22	31.82	18.18	18.18
EB.r3	57,438	81.57	75.08	72.3
EC.r1	37,491	77.11	66.87	64.42
EC.r2	23	26.09	0	0
EC.r3	81,075	76.05	68.26	65.16

Table 1.3. Count data for 16S rRNA read pairs that passed filtering in QIIME2.

Sample	Num. read pairs	% forward reads passing filter	% Non-chimeric forward reads
CA.r1	52,926	79.02	75.61
CA.r2	54,396	77.87	74.52
CA.r3	40,057	80.64	76.84
CB.r1	50,777	76.38	70.98
CB.r2	47,163	77.66	72.73
CB.r3	52,218	75.73	70.36
CC.r1	42,922	74.11	73.65
CC.r2	37,410	76.44	75.09
CC.r3	39,598	72.33	70.88
EA.r1	14,734	65.5	65.31
EA.r2	18,345	65.39	65.23
EA.r3	11,409	64.27	64.02
EB.r1	19,371	73.47	70.23
EB.r2	27,683	66.86	63.72
EB.r3	19,119	69.87	67.39
EC.r1	7,860	65.64	65.14
EC.r2	7,831	60.85	59.09
EC.r3	13,901	61.96	60.61

Table 1.4. Count data for ITS1 read pairs that passed filtering in QIIME2.

Metagenomic sequencing of the six pelagic *Sargassum* tissue samples resulted in approximately 3 billion forward and reverse read pairs with an average of 502 million pairs (Table 1.5). 87.98% (CA), 90.81% (CB), 92.27% (CC), 94.19% (EA), 86.03% (EB), and 89.02% (EC) of the read pairs survived after trimming at a phred score of 15 (Table 1.5). We built individual metagenomic assemblies and co-assemblies using these reads. After binning and dereplicating the MAGs from these samples, we obtained a total of 158 high- and

medium-quality MAGs belonging to nine phyla. The assembly statistics, completion matrices, and the taxonomic information for non-redundant, bacterial MAGs assembled from these samples are listed in Table 1.5. In general, our MAGs had at least 10x coverage with a completion percentage of 50% or greater.

Sample	Num. read pairs	Read pairs trimmed (%)	R1 reads trimmed (%)	R2 reads trimmed (%)
CA	541,489,303	88	11.2	0.58
CB	616,877,205	91	8.3	0.63
CC	488,517,722	92	6.8	0.65
EA	272,077,609	94	5.0	0.55
EB	574,658,998	86	13.1	0.62
EC	522,597,213	89	10.1	0.60

Table 1.5. Count data for metagenomic read pairs that passed quality filters.

1.3.4 Changes in bacterial community composition and diversity

We sequenced the microbial consortia from six whole tissue samples of pelagic *Sargassum* collected from the sea surface aboard the R/V *Meteor* in Spring 2018. Three of these six samples were control samples while the other three were final time point samples collected after 24-hour incubations. After filtering out ASVs assigned to eukaryotes, chloroplast, and mitochondria, 2086 16S rRNA ASVs remained. We found that the relative abundance of bacterial communities at the ASV level differed between the control (CA-CC) and the experimental samples (EA-EC) (ANOSIM statistic R=0.7366, p=0.0002, Figure 1.4A). However, both types of samples shared the same prevalent bacterial taxa.

At the phylum level, the bacterial communities associated with the control and experimental samples mostly comprised of four phyla—*Actinobacteriota*, *Bacteroidota*, *Cyanobacteria*, and *Proteobacteria*. After incubating *Sargassum* tissue for 24 hours, ASVs from *Alteromonadaceae*, *Rhodobacteraceae*, and *Microtrichaceae* families increased in

relative abundance, whereas ASVs of families *Nodosilineaceae*, *Nostocaceae*, *Phormidesmiaceae*, and *Saprospiraceae* decreased in relative abundance (Figure 1.4B).

Alteromonadaceae contains many copiotrophic bacteria that are able to degrade complex sugars (López-Pérez & Rodriguez-Valera, 2014), which may explain their proliferation in bottles with *Sargassum* tissue. As the organic matter accumulated in these bottles over the incubation period, we hypothesize that *Alteromonadaceae* were able to degrade and consume those nutrients, outcompeting other bacteria. Furthermore, members of this taxa have been found on other macroalgae and have been reported to respond positively to macroalgal polysaccharide, agarose (Jain et al., 2020; López-Pérez & Rodriguez-Valera, 2014).

Rhodobacteraceae are primarily photoheterotrophs and are frequently associated with phytoplankton and macroalgal blooms (Jain et al., 2020; Pujalte et al., 2014; Theirlynck et al., 2023). They tend to respond well to high levels of dissolved organic matter which could explain their increase in relative abundance in the presence of *Sargassum*. Lastly, bacteria from the family *Microtrichaceae* (phylum *Actinobacteriota*) have been identified in the epiphytic community of *Sargassum* (Theirlynck et al., 2023), and the uncultivated Sva0996 marine group was the only classified *Microtrichaceae* at the genus level in our dataset. Members of the Sva0996 marine group have been detected in the upper water column of the eastern Pacific Ocean, in sediments of the Arctic Ocean, in marine sponges, and, more recently, in epiphytic communities associated with brown macroalgae (Dat et al., 2018; Ihua et al., 2020; Oppong-Danquah et al., 2023; Orsi et al., 2016; Ravenschlag et al., 1999; Verhoeven et al., 2017). It has been proposed that microorganisms of the Sva0996 marine group are involved in the cycling of dissolved organic nitrogen (Biagi et al., 2020; Orsi et al.,

2016) and possess the ability to potentially degrade complex molecules released by kelp during the later phases of decomposition (Brunet et al., 2021; Prins et al., 2024).

In addition to the community composition and differences, we also estimated the alpha diversity of our samples based on the treatment condition (i.e., control versus experimental). All alpha diversity estimates were significantly lower in the experimental samples compared to the control bottles ($p<0.05$), indicating that the overall biodiversity decreased in the incubated *Sargassum* samples (Figure 1.4C). This decrease in diversity and evenness may be credited to the selective response of certain bacteria to the dissolved organic carbon exuded by *Sargassum*, which allows them to outcompete other species, ultimately dominating a significant portion of the microbial community.

1.3.5 Changes in fungal community composition and diversity

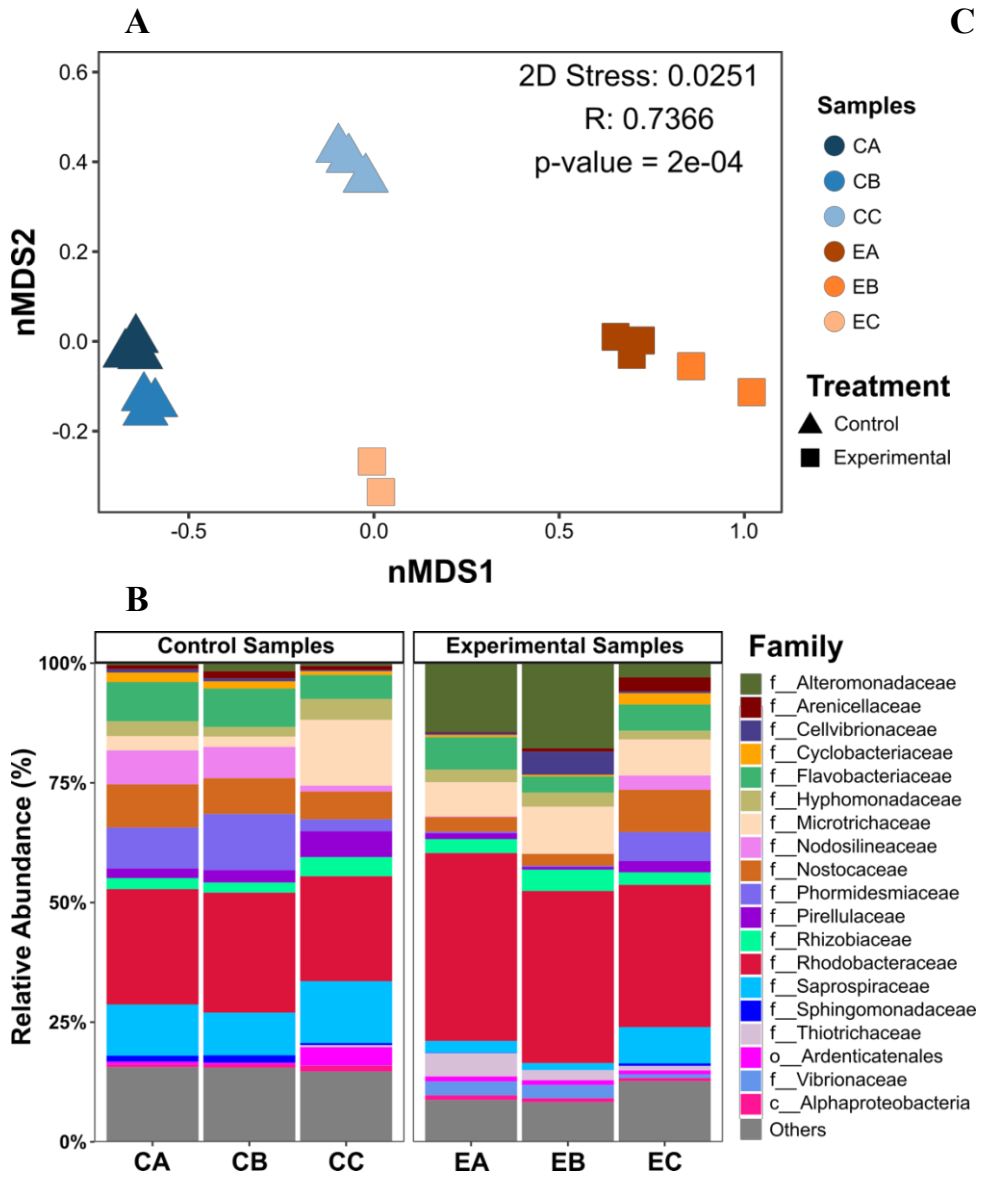
In addition to the 16S rRNA SSU gene, we sequenced the ITS1 gene in order to evaluate the shifts in fungal communities associated with the incubated *Sargassum* samples. We removed all ASVs with an abundance count of zero from our 503 ITS1 amplicons, resulting in a remaining total of 479 ASVs. 61% of the 479 ASVs were unassigned, while 16% were place *incertae sedis* within the kingdom Fungi. Similar to the diversity patterns we observed in the bacterial communities, we found that the relative abundance of fungal communities at the ASV level differed significantly between the control and the experimental samples (ANOSIM statistic $R=0.9331$, $p=0.0002$, Figure 1.5A). This contrast is further supported by the alpha diversity descriptors. In general, the species richness, measured by the Shannon index and the Inverse Simpson index, of the fungal communities associated with *Sargassum* increased after 24 hours of incubation. However, these measures of diversity were not statistically significant. Nonetheless, the increase in richness in the experimental samples was

likely enhanced by an increase in the evenness of the species because the control samples were dominated by a few taxa, and a decline in their prevalence in the incubated tissue samples resulted in a more evenly distributed fungal community. Consequently, the richness indices were positively affected. It is worth noting that although the evenness of the fungal communities in the experimental samples increased, it was not statistically significant ($p>0.05$, Figure 1.5C).

The above alpha and beta diversity differences between control samples and the experimental samples were further supported by the community shifts we observed in the fungal taxa between the treatment types (Figure 1.5A). At the family level, the fungal communities associated with the control samples comprised of unassigned fungi or taxa with uncertain taxonomic placement. However, in the experimental *Sargassum* samples, *Aspergillaceae* was the predominant classified family in all three samples (34%, 3%, and 24%). Overall, the relative abundance of ASVs belonging to the *Aspergillaceae* family increased in the incubated *Sargassum* tissue.

The two predominant genera within the *Aspergillaceae* family identified in our dataset were *Penicillium* and *Aspergillus*, both of which have been discovered in marine environments (A. J. Chen et al., 2017; S. Lee et al., 2016; Y. M. Lee et al., 2010; W. Wang et al., 2018). Both genera harbor fungal members that are known to produce bioactivate compounds (R. Nicoletti & Trincone, 2016). For instance, *Penicillium chrysogenum*, was a fungal strain used to produce the antibiotic penicillin at scale (Gaynes, 2017). Moreover, pertinent to our investigation, members of these genera are known to synthesize siderophores. Wallner et al. 2009 reported that *A. fumigatus* mutant strains, that could not

manufacture ferricrocin and its hydroxylated derivative hydroxyferricrocin, had iron starved conidia (Wallner et al., 2009).



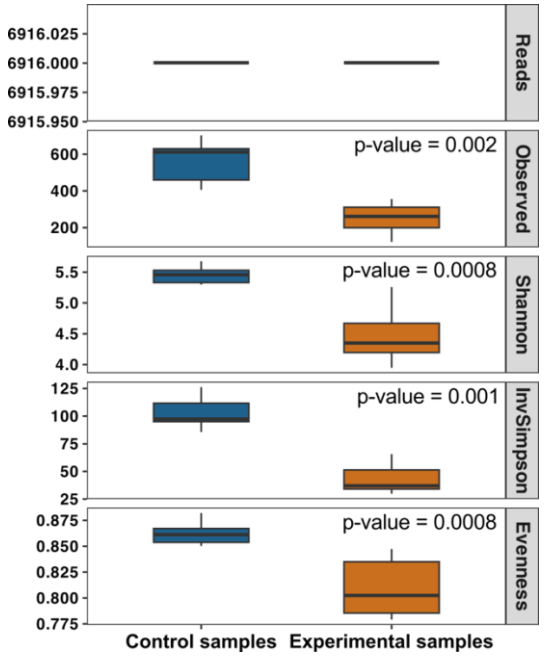


Figure 1.4. (A) Non-metric multi-dimensional scaling plot showing the bacterial community structure of control and experimental *Sargassum* tissue samples. Three technical replicates per biological replicate are shown. The plot was constructed using the Bray-Curtis similarity matrix based on square root transformed relative abundance values. (B) Univariate descriptors of diversity associated with control and experimental samples. All p-values are shown. (C) Relative abundance of bacterial communities associated control and experimental *Sargassum* tissue samples. Taxonomy of the top 20 most abundant families including low abundant taxa grouped into the "others" category.

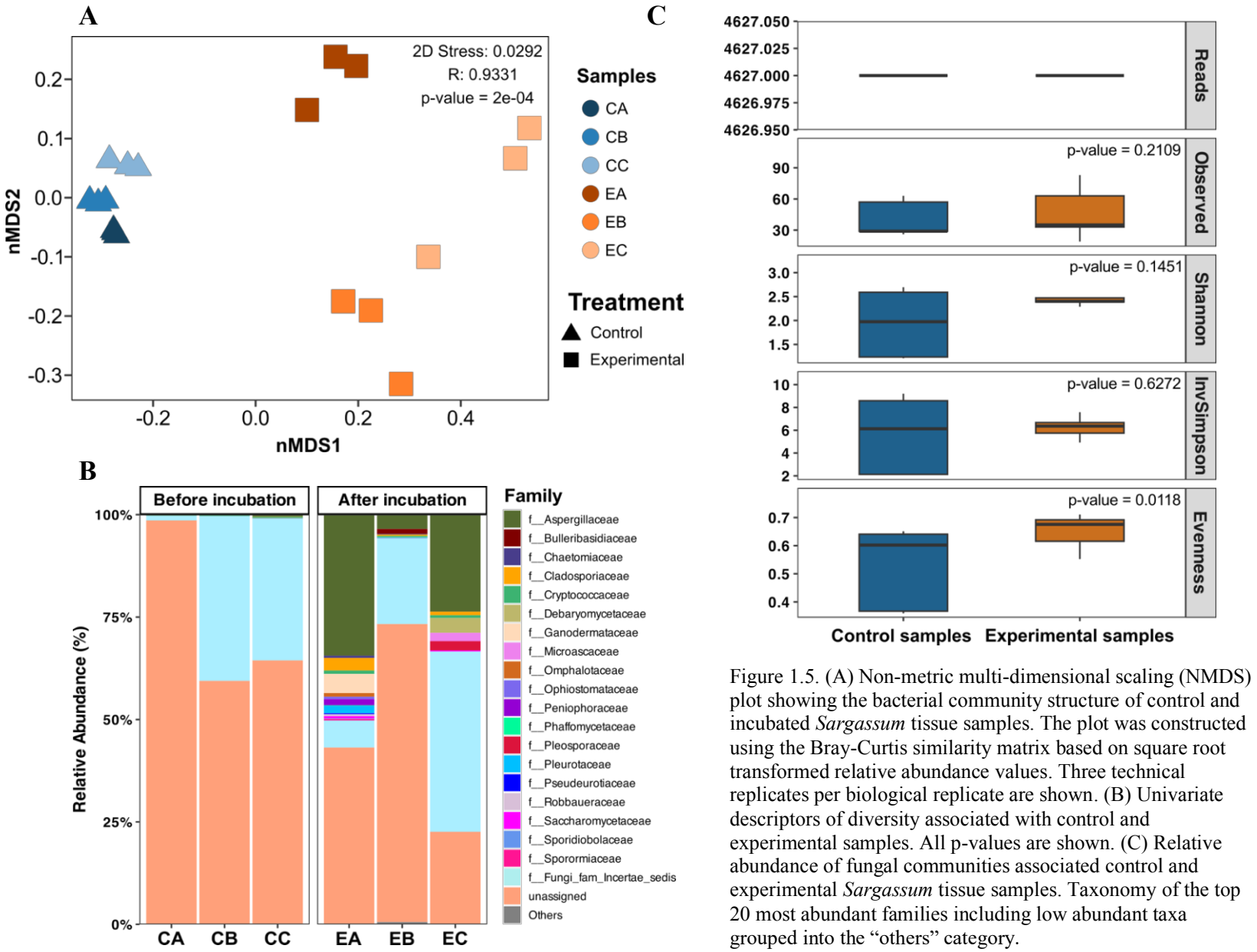


Figure 1.5. (A) Non-metric multi-dimensional scaling (NMDS) plot showing the bacterial community structure of control and incubated *Sargassum* tissue samples. The plot was constructed using the Bray-Curtis similarity matrix based on square root transformed relative abundance values. Three technical replicates per biological replicate are shown. (B) Univariate descriptors of diversity associated with control and experimental samples. All p-values are shown. (C) Relative abundance of fungal communities associated control and experimental *Sargassum* tissue samples. Taxonomy of the top 20 most abundant families including low abundant taxa grouped into the "others" category.

1.3.6 Bacterial communities associated with pelagic *Sargassum* in the tropical north Atlantic Ocean are taxonomically similar

Due to the limited number of samples in our study, we compared our findings with those of other researchers who also studied the microbiome of pelagic *Sargassum*. A comprehensive literature review revealed only one study in which the researchers collected samples from the same region as our study, and whose data were publicly available online (Theirlynck et al., 2023). At the phylum and the family levels, the community composition of our samples (i.e., GEOTRACES M147) differed from those collected by Theirlynck and colleagues (i.e., Atlantic S12, Atlantic S14, Atlantic S24, and Atlantic S28) (Figures 1.6 & 1.7; PERMANOVA, p=0.001).

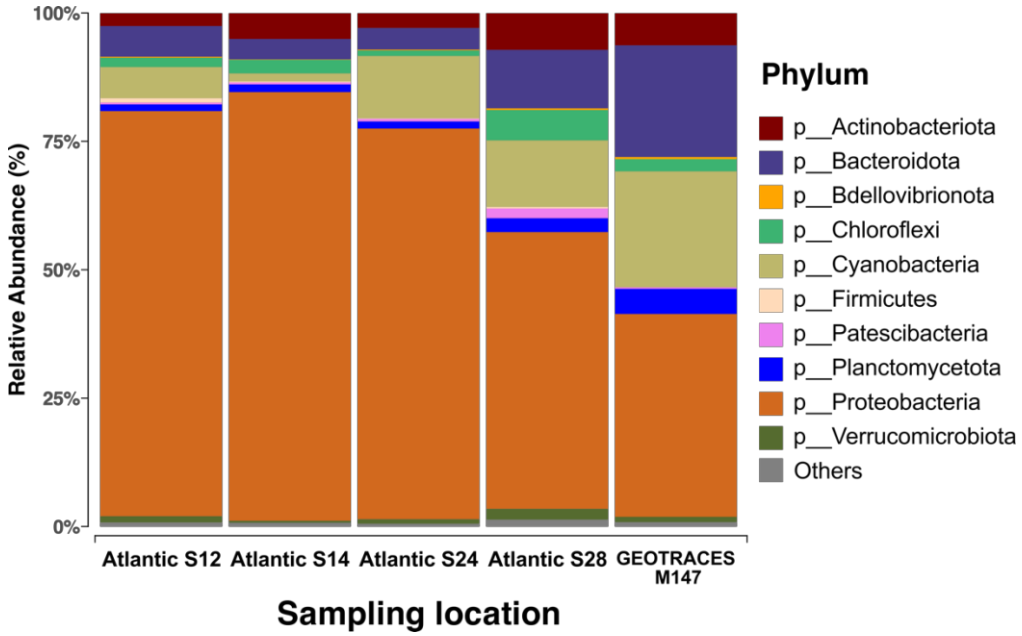


Figure 1.6. Relative abundance of bacterial communities associated with our *Sargassum* tissue samples and those collected by Theirlynck and colleagues. Taxonomy of the top 10 most abundant phyla including low abundant taxa grouped into the "others" category. Atlantic S12 through Atlantic S28 are samples from the Theirlynck study and GEOTRACES M147 represents this study's control samples.

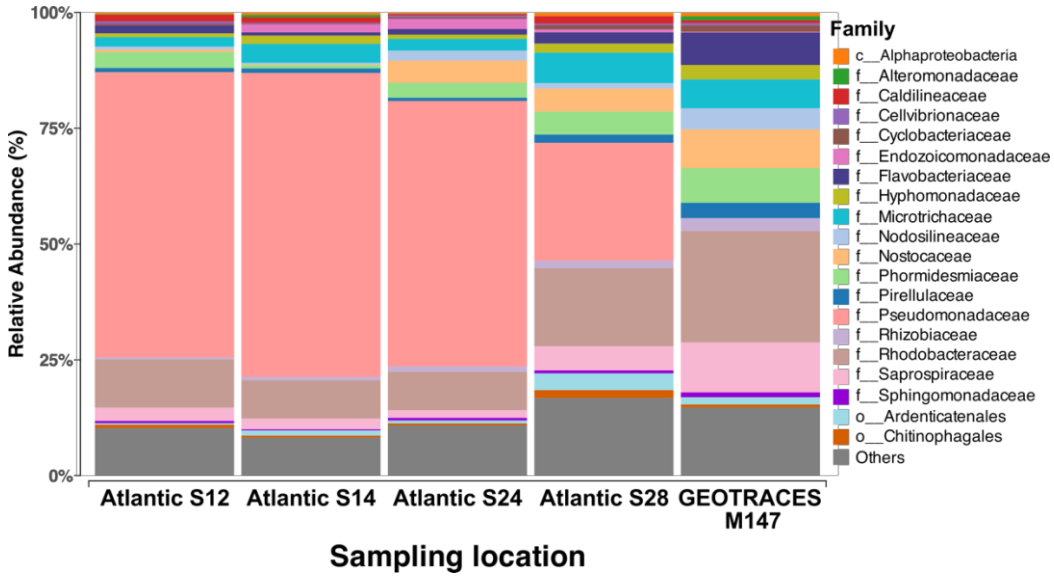


Figure 1.7. Relative abundance of bacterial communities associated with our *Sargassum* tissue samples and those collected by Theirlynck and colleagues. Taxonomy of the top 20 most abundant families including low abundant taxa grouped into the "others" category. Atlantic S12 through Atlantic S28 are samples from the Theirlynck study and GEOTRACES M147 represents this study's control samples.

At the family level, members of the *Pseudomonadaceae* family, all belonging to the *Pseudomonas* genus, were abundant in the other study's samples and absent in ours. This family likely drove the community differences we observed between our and Theirlynck's samples. Despite this, our and Theirlynck's samples share several bacterial families, indicating that pelagic *Sargassum* associates with consistent bacterial taxa (Figure 1.7).

1.3.7 Microbial communities associated with pelagic *Sargassum* are mainly composed of heterotrophic bacteria

In addition to 16S rRNA, we sequenced the metagenomes of *Sargassum's* microbial associates and built a set of 158 nonredundant metagenome-assembled genomes. These genomes were assigned to nine bacterial phyla, with *Pseudomonadota* representing 73% of the MAGs (Table 1.6 & Figure 1.8). After *Pseudomonadota*, 11% and 5% of the MAGs were classified into phyla *Bacteroidota* and *Cyanobacteriota*, respectively (Figure 1.8). In order to

gain insights into their metabolic capabilities, we used METABOLIC v4.0 to generate biochemical profiles of our MAGs. Many bacteria associated with *Sargassum* lacked the ability to perform carbon fixation and are primarily heterotrophic and photoheterotrophic in nature (Figure 1.9). According to our metabolic profiles of the MAGs, nearly all bacteria are unable to fix carbon to generate their own energy and likely rely on *Sargassum* to fulfill their carbon needs. Exceptions include members of the *Cyanobacteriota* phylum that can fix carbon as well as nitrogen (Figure 1.10). The mechanisms of nitrogen metabolism in the *Sargassum*-associated cyanobacteria are discussed further in the following section 1.3.8.

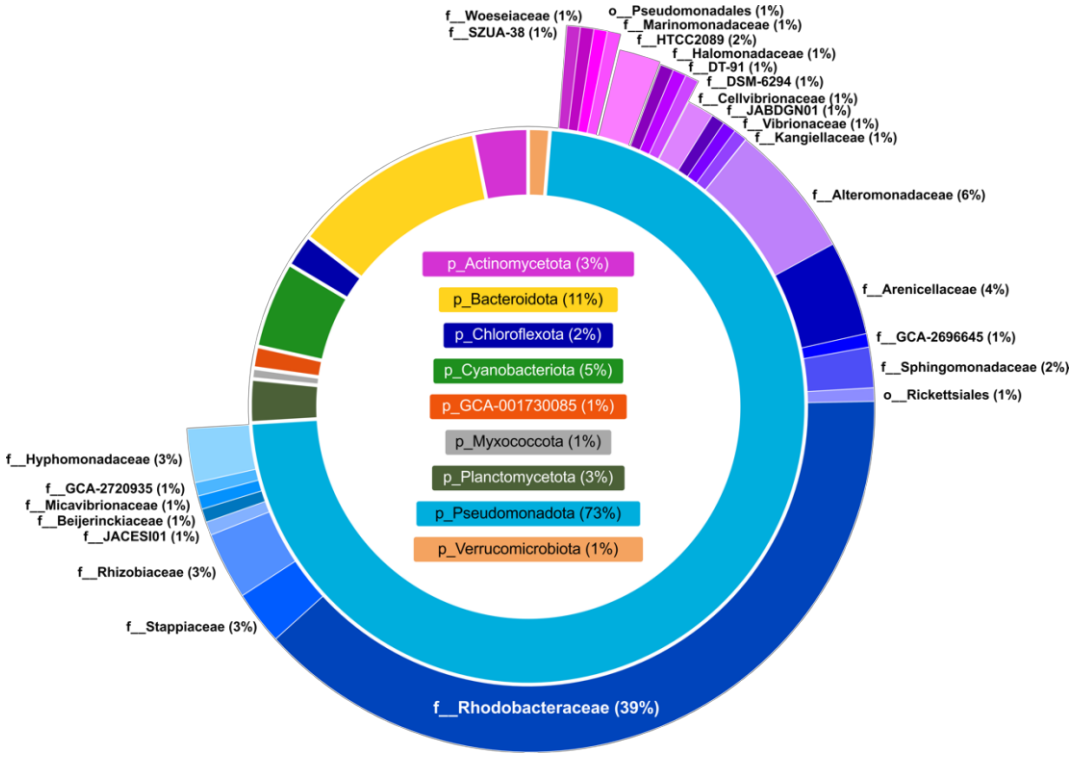


Figure 1.8. Taxonomic distribution of the 158 dereplicated metagenome-assembled genomes (MAGs) based on GTDB-Tk v2.3.0 (Genome Taxonomy Database R214) classification. The phyla and their respective percentages are displayed in the inner circle and the families of *Pseudomonadota* phylum are shown in the outer circle.

MAG ID	Comp.	Cont.	Cov. (x)	Contig num.	Genome size (bp)	Longest contig (bp)	N50 (bp)	GC (%)	Phylum	Class	Order	Family	Genus	Species
EAEBEC.53	95.3	2.75	22.16	200	3290332	89142	25017	60.39	p__Actinomycetota	c__Acidimicrobiia	o__Acidimicrobiales	f__SHLQ01	g__SHLQ01	-
all.Sarg.132	59.22	5.64	10.23	641	3434459	26246	5872	64.04	p__Actinomycetota	c__Acidimicrobiia	o__Acidimicrobiales	f__SZUA-35	-	-
all.Sarg.221	97.15	5.12	15.10	276	4970701	95462	25074	68.61	p__Actinomycetota	c__Acidimicrobiia	o__Acidimicrobiales	f__SZUA-35	g__CADEDH01	g__CADEDH01
EAEBEC.216	91.53	2.56	15.30	329	6036542	116166	27228	67.1	p__Actinomycetota	c__Acidimicrobiia	o__Acidimicrobiales	f__UBA10347	g__UBA10347	g__UBA10347
all.Sarg.103	76.05	2.64	12.59	441	3360338	36558	9544	63.86	p__Actinomycetota	c__Acidimicrobiia	o__Acidimicrobiales	f__UBA10347	g__UBA10347	g__UBA10347
all.Sarg.143	73.72	1.6	10.56	511	3680900	28871	7953	45.73	p__Bacteroidota	c__Bacteroidia	o__Chitinophagales	f__Saprospiraceae	-	-
EAEBEC.71	50.9	3.63	8.11	932	3961485	16738	4335	37.41	p__Bacteroidota	c__Bacteroidia	o__Chitinophagales	f__Saprospiraceae	g__JAAUTG01	g__JAAUTG01
all.Sarg.280	51.07	0.5	11.51	836	3980427	19857	5050	40.76	p__Bacteroidota	c__Bacteroidia	o__Chitinophagales	f__Saprospiraceae	g__JAHDEO01	g__JAHDEO01
all.Sarg.295	56.03	3.45	11.75	641	3606838	23963	6268	38.05	p__Bacteroidota	c__Bacteroidia	o__Chitinophagales	f__Saprospiraceae	g__JAHDFB01	g__JAHDFB01
EB.90	98.27	1.98	41.33	338	8673078	165089	41396	39.91	p__Bacteroidota	c__Bacteroidia	o__Chitinophagales	f__Saprospiraceae	g__JALZUU01	g__JALZUU01
EC.37	88.31	3.76	9.82	584	3899450	45266	8304	38.97	p__Bacteroidota	c__Bacteroidia	o__Cytophagales	f__Cyclobacteriaceae	g__Ekhidna	g__Ekhidna
EAEBEC.128	90.42	2.23	25.34	401	4381808	54453	14940	38.56	p__Bacteroidota	c__Bacteroidia	o__Cytophagales	f__Cyclobacteriaceae	g__Fabibacter	g__Fabibacter
all.Sarg.195	96.5	1.93	23.09	110	3564186	175024	47348	43.68	p__Bacteroidota	c__Bacteroidia	o__Cytophagales	f__Cyclobacteriaceae	g__Reichenbachiella	g__Reichenbachiella
EC.94	92.82	0.91	33.93	193	4486475	121132	38337	45.52	p__Bacteroidota	c__Bacteroidia	o__Cytophagales	f__Flammeovirgaceae	-	-
EAEBEC.44	89.22	2.97	11.19	573	4678364	49324	10063	36.12	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Crocinitomicaceae	g__Crocinitomix	g__Crocinitomix
EC.158	97.46	2.52	13.07	579	4316701	50465	9379	33.5	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Aquimarina	g__Aquimarina
EAEBEC.50	93.21	1.75	15.41	477	4453630	58521	12418	33.64	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Aquimarina	g__Aquimarina
EAEBEC.60	89.92	1.39	17.04	289	2989371	48328	13516	35.38	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Dokdonia	g__Dokdonia
all.Sarg.104	65.52	0	10.30	337	2283852	33959	7845	42.2	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Gilvibacter	g__Gilvibacter
EB.143	100	1.88	26.99	118	5423907	175076	66695	34.16	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Kordia	g__Kordia
EC.41	98.01	1.2	17.16	179	3784490	116486	31977	31.04	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Tenacibaculum	g__Tenacibaculum
EC.42	94.8	1.27	42.36	382	3952656	49921	14213	30.48	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Tenacibaculum	g__Tenacibaculum
all.Sarg.279	97.47	1.37	14.62	421	8495100	101303	29877	47.46	p__Bacteroidota	c__Bacteroidia	o__J057	f__J057	g__JAGQVA01	g__JAGQVA01
EB.132	61.97	3.64	13.57	528	2967168	22106	6009	48.37	p__Chloroflexota	c__Anaerolineae	o__Promineofilales	f__Promineofilaceae	g__JACKBM01	g__JACKBM01
EAEBEC.194	80.76	3.82	16.33	606	4864227	50940	9449	52.74	p__Chloroflexota	c__Anaerolineae	o__Promineofilales	f__Promineofilaceae	g__JAMLHW01	g__JAMLHW01
all.Sarg.190	92.68	4.27	11.97	594	4823592	34572	10379	55.18	p__Chloroflexota	c__Anaerolineae	o__Promineofilales	f__Promineofilaceae	g__JAMLHW01	g__JAMLHW01
all.Sarg.235	86.81	0.22	19.14	681	5120540	50762	9149	36.39	p__Cyanobacteriota	c__Cyanobacteriia	o__Cyanobacteriales	f__Nostocaceae	g__RDXV01	s__RDXV01 sp010672785
CA.36	93.52	2.26	32.35	706	7144639	62992	13054	37.09	p__Cyanobacteriota	c__Cyanobacteriia	o__Cyanobacteriales	f__Nostocaceae	g__Rivularia	g__Rivularia
CACBCC.110	91.59	3.11	18.97	787	7196077	58207	11270	36.99	p__Cyanobacteriota	c__Cyanobacteriia	o__Cyanobacteriales	f__Nostocaceae	g__Rivularia	g__Rivularia
EAEBEC.12	69.96	1.95	10.10	627	4540403	38117	8120	47.63	p__Cyanobacteriota	c__Cyanobacteriia	o__Elainellales	f__Elainellaceae	g__JAAHGY01	g__JAAHGY01
EAEBEC.89	63.54	3.99	15.13	704	4766222	29687	7318	48.77	p__Cyanobacteriota	c__Cyanobacteriia	o__Elainellales	f__Elainellaceae	g__JAAHGY01	g__JAAHGY01
EB.53	91.26	2.72	59.95	585	6570709	58177	16193	48.15	p__Cyanobacteriota	c__Cyanobacteriia	o__Phormidesmiales	f__Phormidesmiaceae	g__Leptothoe	g__Leptothoe
CACBCC.81	85.55	1.4	14.85	467	4187440	41212	11203	53.58	p__Cyanobacteriota	c__Cyanobacteriia	o__Phormidesmiales	f__Phormidesmiaceae	g__PCC-6406	g__PCC-6406
all.Sarg.207	91.94	2.49	26.99	380	4991041	112526	18240	53.34	p__Cyanobacteriota	c__Cyanobacteriia	o__Phormidesmiales	f__Phormidesmiaceae	g__PCC-6406	g__PCC-6406
CACBCC.19	95.16	0	21.77	171	1335954	41956	9538	35.43	p__GCA-001730085	c__GCA-001730085	o__GCA-001730085	f__GCA-001730085	g__GCA-001730085	s__GCA-001730085 sp001730085
all.Sarg.184	97.85	0	26.36	141	1483686	57469	13684	35.12	p__GCA-001730085	c__GCA-001730085	o__GCA-001730085	f__GCA-001730085	g__GCA-001730085	s__GCA-001730085 sp001730085
all.Sarg.249	67.71	0.97	11.08	1171	6333852	28217	5916	64.44	p__Myxococcota	c__Polyangia	o__Polyangiales	f__SG8-38	-	-
all.Sarg.125	94.83	0.59	23.21	216	5565550	150602	37866	51.67	p__Planctomycetota	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	g__Mariniblastus	g__Mariniblastus
EAEBEC.193	66.86	2.33	9.85	809	4076149	25934	5388	70.89	p__Planctomycetota	c__UBA1135	o__UBA1135	f__GCA-002686595	g__Pla163	g__Pla163
EC.117	62.17	5.95	8.76	871	4250140	24415	5207	68.79	p__Planctomycetota	c__UBA1135	o__UBA2386	f__UBA2386	-	-
all.Sarg.88	86.97	9.14	14.75	502	5874290	67899	16166	68.61	p__Planctomycetota	c__UBA1135	o__UBA2386	f__UBA2386	-	-
all.Sarg.148	88.59	7.34	13.71	315	3012447	40455	13154	53.08	p__Pseudomonadota	c__Alphaproteobacteria	o__Caulobacterales	f__Hyphomonadaceae	-	-
EB.68	93.46	3.23	21.87	341	3429359	71099	14663	55.06	p__Pseudomonadota	c__Alphaproteobacteria	o__Caulobacterales	f__Hyphomonadaceae	g__Henriciella	g__Henriciella
EB.140	89.32	4.71	25.80	410	3735701	66734	11865	54.97	p__Pseudomonadota	c__Alphaproteobacteria	o__Caulobacterales	f__Hyphomonadaceae	g__Henriciella	g__Henriciella
all.Sarg.285	65	1.72	11.63	447	2935809	35956	7378	54.77	p__Pseudomonadota	c__Alphaproteobacteria	o__Caulobacterales	f__Hyphomonadaceae	g__Henriciella	g__Henriciella
EC.107	56.27	0.54	8.63	223	1109397	18025	5163	36.72	p__Pseudomonadota	c__Alphaproteobacteria	o__Micavibrionales	f__GCA-2720935	-	-
EC.138	89.41	1.01	11.84	135	1740073	51251	19558	46.61	p__Pseudomonadota	c__Alphaproteobacteria	o__Micavibrionales	f__Micavibrionaceae	g__JAGRKE01	g__JAGRKE01
all.Sarg.155	75.88	1.46	12.43	682	3649017	23841	5835	71.06	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Beijerinckiaceae	g__Methylobacterium	s__Methylobacterium jeotgali
all.Sarg.30	69.12	3.79	10.73	418	2880974	39797	8135	49.24	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__JACESI01	g__JACESI01	g__JACESI01
all.Sarg.172	95.99	1.67	18.39	119	3008420	141672	42826	52.3	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	-	-
CACBCC.16	93.91	0.9	19.19	125	2415714	78231	29013	52.19	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	g__Ahrensia	g__Ahrensia
CACBCC.49	98.39	0.76	35.35	138	3213896	112564	31934	48.28	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	g__GCA-2401155	g__GCA-2401155
EB.130	72.41	6.9	18.00	538	3767588	37249	8308	45.45	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	g__Lentilitoribacter	g__Lentilitoribacter
all.Sarg.237	97.36	0.78	23.66	55	3232113	315533	104182	53.28	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Rhizobiaceae	g__Salaquimonas	g__Salaquimonas
CB.3	94.05	3.7	17.46	752	6055773	47853	10190	58.69	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Stappiaceae	g__Roseibium	g__Roseibium
EB.7	76.54	9.48	18.56	618	5946219	57165	12061	58.85	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Stappiaceae	g__Roseibium	g__Roseibium
EB.39	95.16	6.53	22.78	527	6347318	73479	16822	58.57	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Stappiaceae	g__Roseibium	g__Roseibium
EC.93	98.5	3.84	32.96	306	5311754	142227	25932	54.22	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Stappiaceae	g__Roseibium	g__Roseibium
CA.60	95.32	3.22	17.86	193	3938169	112158	35966	62.93	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	-	-
EB.6	92.03	6.16	16.99	467	6052051	79821	20588	57.56	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	-	-
EB.40	72.66	2.91	29.51	425	2579516	37569	6882	58.12	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	-	-
EC.113	90.78	3.37	70.78	359	3514564	85940	12549	58.04	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	-	-
EAEBEC.103	88.42	3.86	41.83	254	3261899	88525	17440	59.48	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	-	-
EAEBEC.109	92.02	1.23	13.85	257	4102845	105569	23002	60.09	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	-	-
EAEBEC.151	87.29	4.9	17.45	201	3489505	83661	26426	60.27	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	-	-
all.Sarg.201	92.8	0.8	27.74	40	3587098	369341	190671	56.72	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	-	-

MAG ID	Comp.	Cont.	Cov. (x)	Contig num.	Genome size (bp)	Longest contig (bp)	N50 (bp)	GC (%)	Phylum	Class	Order	Family	Genus	Species
all.Sarg.246	85.34	0	21.90	139	3497380	120415	50185	60.44	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	-	-
EB.125	66.23	5.78	11.06	717	3646030	25762	5454	58.25	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__188UL27-1	g__188UL27-1
EAEBEC.170	94.8	4.5	19.78	370	4469750	148058	17009	61.41	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__188UL27-1	g__188UL27-1
all.Sarg.130	85.89	8.48	22.41	147	3186725	114747	37770	56.59	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Aliishimia	g__Aliishimia
CB.31	98.52	2.21	20.46	144	3373695	125069	47716	52.14	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Amylibacter	g__Amylibacter
EC.10	97.01	1.11	16.50	104	3361787	166511	56539	55.64	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Amylibacter	g__Amylibacter
EC.12	95.45	1.26	98.13	91	2817009	118057	54007	47.77	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Amylibacter	g__Amylibacter
EC.44	95.61	0.35	14.49	106	2873097	148092	48481	48.47	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Amylibacter	g__Amylibacter
all.Sarg.80	97.92	1.33	95.55	75	3425637	248612	98104	52.16	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Amylibacter	g__Amylibacter
all.Sarg.282	55.17	3.45	15.58	465	2500085	75133	5815	63.36	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Amylibacter	g__Amylibacter
EC.74	68.92	1.72	9.82	443	2407156	17260	5981	50.47	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Celeribacter	g__Celeribacter
EA.11	83.66	6.63	21.76	814	2650459	18502	3929	54.92	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Cognatishimia	g__Cognatishimia
EC.2	70.59	6.14	13.62	569	3094723	44542	6003	54.9	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Cognatishimia	g__Cognatishimia
CB.11	91.98	1.65	16.19	258	3347383	109236	21700	55.17	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Cognatiyoonia	g__Cognatiyoonia
EB.62	54.97	0	13.90	317	2285902	32292	8345	55.21	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Cognatiyoonia	g__Cognatiyoonia
EB.106	95.73	3.53	24.09	117	3860808	280832	69172	54.53	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Epibacterium	g__Epibacterium
EB.109	94.88	2.52	28.08	176	3996997	151855	34840	58.28	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Epibacterium	g__Epibacterium
EC.25	82.52	3.99	16.84	466	3376702	42444	8576	56.91	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Epibacterium	g__Epibacterium
EAEBEC.180	76.36	6.9	78.01	264	3453822	88799	17220	55.71	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Epibacterium	g__Epibacterium
EB.30	96.04	1.45	33.00	185	3551441	128551	30196	60.47	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__HKCCE3408	g__HKCCE3408
all.Sarg.299	76.82	5.39	22.13	267	3251645	61506	17085	59.32	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__IMCC34051	g__IMCC34051
CACBCC.104	96.22	1.27	24.15	72	3248054	214668	68825	61.81	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__JAIVHI01	g__JAIVHI01
EC.145	66.86	3.85	9.46	527	2647280	40481	5479	60.92	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__JAKVBH01	g__JAKVBH01
CACBCC.9	97.88	1.92	30.32	101	3836382	288809	57556	56.82	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__JAKVBH01	g__JAKVBH01
all.Sarg.83	81.26	8.73	11.23	500	3084124	30475	6970	58.52	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Kangsaoukella	g__Kangsaoukella
EC.81	98.09	2	15.31	142	3846181	157620	54580	58.64	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Litoreibacter	g__Litoreibacter
EC.133	58.5	5.67	11.09	594	2912793	24359	5302	59.47	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Mangrovicoccus	g__Mangrovicoccus
EC.143	91.67	4.39	19.90	236	3611556	90975	23009	52.51	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pelagimonas	g__Pelagimonas
EC.105	93.85	1.5	18.26	202	4385283	115925	34525	65.95	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pikeienuella	g__Pikeienuella
EC.124	94.34	4.49	15.11	361	4470098	48856	17842	69.2	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pikeienuella	g__Pikeienuella
CACBCC.13	96.52	2.13	25.83	133	4223696	142342	48052	66.54	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pikeienuella	g__Pikeienuella
EAEBEC.168	52.3	5.17	11.33	515	2589333	21944	5328	58.71	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pikeienuella	g__Pikeienuella
all.Sarg.68	89.61	1	27.74	49	3909412	353452	137914	59.44	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pikeienuella	g__Pikeienuella
all.Sarg.218	98.51	2.6	34.87	72	4590820	323368	105014	69.12	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pikeienuella	g__Pikeienuella
all.Sarg.294	92.05	4.46	24.81	75	3927244	260880	98052	65.03	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pikeienuella	g__Pikeienuella
EC.111	80.62	2.42	23.52	472	3452981	38052	9101	64.29	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pseudaestuariivita	g__Pseudaestuariivita
EB.9	94.26	6.81	13.40	267	3437778	62285	20902	58.28	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pseudooctadecabacter	g__Pseudooctadecabacter
all.Sarg.96	84.15	3.72	35.44	68	2879190	194269	78436	58.79	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pseudooctadecabacter	g__Pseudooctadecabacter
EAEBEC.95	58.26	1.72	11.72	572	3138892	23596	6127	61.99	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Pseudophaeobacter_A	s__Pseudophaeobacter_A gallaeciensis_A
all.Sarg.9	76.54	8.17	19.10	99	3005957	120102	56883	58.68	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Roseovarius	g__Roseovarius
all.Sarg.120	95.73	4.67	17.43	152	3667564	175589	38710	55.7	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Roseovarius	g__Roseovarius
all.Sarg.64	68.47	5.71	14.59	227	2296855	53757	15367	61.99	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Salibaculum	g__Salibaculum
CB.7	97.92	2.08	44.14	147	4484912	163141	44464	57.63	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Tateyamaria	g__Tateyamaria
EC.27	91.5	3.66	13.35	530	6227752	87035	17270	59.74	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Thermohalobaculum	g__Thermohalobaculum
EC.50	98.3	1.53	67.04	297	4172295	73743	19712	60.49	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Thermohalobaculum	g__Thermohalobaculum
all.Sarg.194	74.05	6.09	10.92	820	4544658	23864	6342	63.43	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__WNWL01	g__WNWL01
EB.65	61.49	2.52	9.46	487	2421104	24072	5234	57.81	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__WX04	g__WX04
all.Sarg.79	80.85	4.72	18.32	251	2614475	59426	14350	63.64	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__WX04	g__WX04
all.Sarg.174	79.09	6.33	21.41	201	2930016	62056	21730	57.89	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__WX04	g__WX04
CB.33	77.92	2.62	12.51	470	2975687	32369	7269	59.55	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Yoonia	g__Yoonia
EC.9	82.77	4.92	25.42	672	4494730	43420	7854	56.3	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Yoonia	g__Yoonia
EAEBEC.86	89.91	7.37	19.80	130	3628947	234185	78155	58.98	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Yoonia	g__Yoonia
EAEBEC.102	93.62	0.41	33.59	54	3267242	249878	110883	55.77	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Yoonia	g__Yoonia
EC.100	100	0	20.58	30	1604815	163466	77321	46.67	p__Pseudomonadota	c__Alphaproteobacteria	o__Rickettsiales	-	-	-
CA.34	96.03	2.99	16.34	203	3005848	57695	22694	57.07	p__Pseudomonadota	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Erythrobacter	g__Erythrobacter

MAG ID	Comp.	Cont.	Cov. (x)	Contig num.	Genome size (bp)	Longest contig (bp)	N50 (bp)	GC (%)	Phylum	Class	Order	Family	Genus	Species
EB.27	95.85	2.23	19.99	216	3313372	107641	25778	54.69	p__Pseudomonadota	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Erythrobacter	g__Erythrobacter
EC.146	91.68	3.26	16.08	305	3461450	67932	15314	55.58	p__Pseudomonadota	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__Erythrobacter	g__Erythrobacter
EC.86	92.47	0	56.13	61	3765657	216641	106917	41.84	p__Pseudomonadota	c__Alphaproteobacteria	o__UBA8366	f__GCA-2696645	g__Curvivirga	g__Curvivirga
CB.23	61	1.02	38.58	435	1853087	14945	4390	41.33	p__Pseudomonadota	c__Gammaproteobacteria	o__Arenicellales	f__Arenicellaceae	-	-
EAEBEC.80	86.18	0.2	41.20	279	2970412	61519	13535	40.65	p__Pseudomonadota	c__Gammaproteobacteria	o__Arenicellales	f__Arenicellaceae	-	-
all.Sarg.69	86.08	4.47	15.59	287	2503958	41057	10846	41.3	p__Pseudomonadota	c__Gammaproteobacteria	o__Arenicellales	f__Arenicellaceae	-	-
EC.19	97.56	0.61	25.39	62	3579042	442344	137937	51.19	p__Pseudomonadota	c__Gammaproteobacteria	o__Arenicellales	f__Arenicellaceae	g__Arenicella	g__Arenicella
EC.151	52.09	0.61	10.86	480	2240547	37640	4727	45.48	p__Pseudomonadota	c__Gammaproteobacteria	o__Arenicellales	f__Arenicellaceae	g__Arenicella	g__Arenicella
EAEBEC.155	95.62	0.91	25.55	147	3237944	123928	31648	45.3	p__Pseudomonadota	c__Gammaproteobacteria	o__Arenicellales	f__Arenicellaceae	g__Arenicella	g__Arenicella
all.Sarg.129	81.03	1.22	12.75	234	2743283	86183	16881	45.58	p__Pseudomonadota	c__Gammaproteobacteria	o__Arenicellales	f__Arenicellaceae	g__Arenicella	g__Arenicella
EC.54	76.27	1.01	9.39	591	3502954	23002	6687	45.86	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	-	-
EC.69	95.9	0.74	24.97	90	4984913	193567	100375	44.61	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	-	-
EAEBEC.199	98.99	1.59	41.38	16	4536483	1338531	542810	44.18	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	-	-
all.Sarg.233	96	1.5	17.58	315	4161483	76843	18886	50.37	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	g__Aestuariibacter_A	g__Aestuariibacter_A
EC.52	88.86	3.57	12.47	318	4477147	68944	19031	45.65	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	g__Alteromonas	g__Alteromonas
EC.110	95.3	2.81	18.61	78	4501406	358921	119589	45.51	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	g__Alteromonas	g__Alteromonas
EC.125	67.55	0	132.62	360	3630133	90389	14309	41.75	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	g__Alteromonas	g__Alteromonas
all.Sarg.106	89.02	5.79	13.83	440	4910909	127935	16801	42.82	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	g__Alteromonas_E	g__Alteromonas_E
all.Sarg.54	63.2	1.51	9.32	811	4188270	41911	5358	42.63	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	g__JABSOG01	g__JABSOG01
EC.88	98.09	3.16	38.36	181	5716799	166871	58411	42.22	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	g__M269	g__M269
EC.53	99.82	0.81	14.80	251	4385594	99715	30104	37.79	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Kangiellaceae	g__Aliikangiella	g__Aliikangiella
all.Sarg.94	89.51	3.45	13.58	362	3182381	43725	11018	44.46	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Vibrionaceae	g__Vibrio	s__Vibrio metschnikovii
CACBCC.85	94.34	0	14.60	128	1651522	94034	17572	41.47	p__Pseudomonadota	c__Gammaproteobacteria	o__JABDGN01	f__JABDGN01	-	-
EC.33	67.24	1.72	18.96	379	2625346	37996	8199	44.45	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	-	-	-
EB.133	96.13	1.33	13.13	388	4629665	65935	17843	43.84	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Cellvibrionaceae	-	-
EC.62	97.97	1.44	21.47	111	3251010	176958	42432	45.49	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Cellvibrionaceae	g__Agaribacterium	g__Agaribacterium
EC.103	66.54	1.36	8.12	434	2243775	27059	5367	48.41	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__DSM-6294	g__Oleispira	g__Oleispira
EC.134	99.51	0	24.68	92	3424542	170974	64009	48.5	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__DT-91	g__DT-91	g__DT-91
CC.2	95.71	1.54	53.27	732	4869538	90300	8574	52.65	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Halomonadaceae	g__Halomonas	s__Halomonas hamiltonii
all.Sarg.26	91	2.08	20.80	173	4282358	247066	42383	61.86	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	-	-
all.Sarg.261	90.96	3.43	23.77	250	4870668	171563	40965	68.2	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	-	-
EC.45	87.58	3.73	21.46	343	4011667	87681	19035	63.21	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	g__JANSWO01	g__JANSWO01
EC.8	97.42	2.48	186.79	99	3396063	203648	55435	41.52	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Marinomonadaceae	g__Marinomonas	g__Marinomonas
all.Sarg.45	85.86	4.89	13.07	319	3398444	66466	13266	55.37	p__Pseudomonadota	c__Gammaproteobacteria	o__Woeseiales	f__Woeseiaceae	-	-
all.Sarg.116	88.02	4.95	16.03	485	3366552	33662	8134	55.16	p__Pseudomonadota	c__Gammaproteobacteria	o__Xanthomonadales	f__SZUA-38	g__SZUA-38	g__SZUA-38
EB.22	78.64	1.35	12.17	429	2556030	21918	6558	50.52	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__JABSRG01	g__JABSRG01	g__JABSRG01
all.Sarg.213	96.46	2.7	21.19	281	4157272	89539	20182	48.25	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__JABSRG01	g__JABSRG01	g__JABSRG01

Table 1.6. Assembly statistics, completion matrices, and the taxonomic information for non-redundant metagenomic assembled genomes (MAGs). Assembly and completion matrices were generated using CheckM v1.2.2 , and the taxonomy was assigned with GTDB-Tk v2.3.0 using the Genome Taxonomy Database R214.

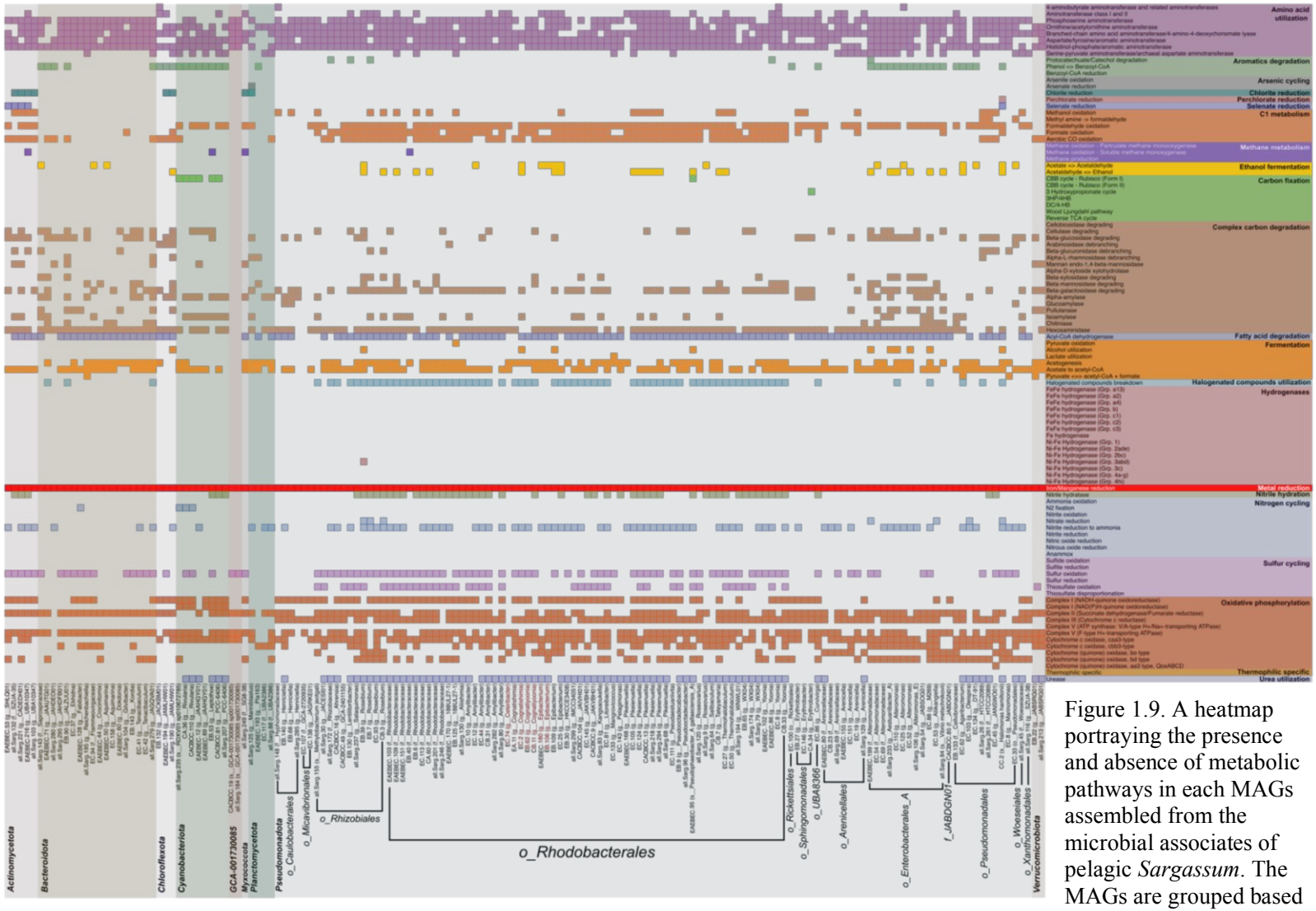
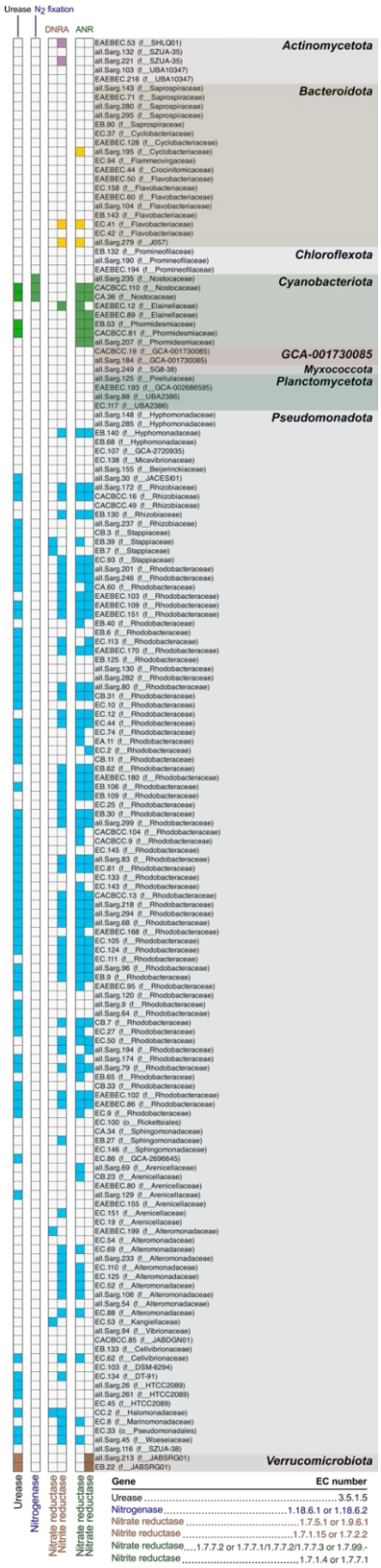


Figure 1.9. A heatmap portraying the presence and absence of metabolic pathways in each MAGs assembled from the microbial associates of pelagic *Sargassum*. The MAGs are grouped based phylum-level classification and members of the *Pseudomonadota* phylum are further categorized into bacterial orders.

1.3.8 Microbes associated with pelagic *Sargassum* can fix nitrogen and remineralize various forms of nitrogen species

As stated previously, we observed an increase in nitrite concentration from below detection (0.01 µmol L^{-1}) in the control samples at the start of the incubation period to 0.564 ± 0.022 µmol L^{-1} in the final samples at the end of the incubation period (Figure 1.3). Nitrite is an intermediate in various pathways such as denitrification, dissimilatory and assimilatory nitrate reduction, and nitrification (Hutchins & Capone, 2022). The presence of nitrite in our bottles suggests that certain bacteria associated with *Sargassum* may be able to perform one or more of these processes. However, both dissimilatory nitrate reduction and denitrification typically occur under anoxic conditions and the latter process is generally known to begin when O_2 concentrations fall below 2-4 µM (Jürgens & Taylor, 2018). Nevertheless, there is substantial scientific evidence that disputes this minimum threshold. For instance, a study conducted by Gao and colleagues (2010) found that denitrification can take place at oxygen



concentrations as high as 90 μM in marine sediments of the Wadden Sea (Gao et al., 2010). Moreover, Dalsgaard et al., (2014) observed only 50% inhibition in denitrification at oxygen concentrations as high as 205 nM in oxygen minimum zones off Northern Chile (Dalsgaard et al., 2014). Extending beyond these findings, our own data reveal that five microbial taxa associated with *Sargassum* within the *Pseudomonadota* phylum possess the capability to catalyze the first step of denitrification, which involves the reduction of nitrate to nitrite. This initial step is shared with the DNRA pathway, as illustrated in Figure 1.10. Alternatively, the observed nitrite may be a product of nitrification which could explain its presence in our incubated bottles. This process typically results in the accumulation of nitrite in oxygen minimum zones but past investigations have shown evidence of nitrification in the euphotic layer (Wankel et al., 2007). Therefore, it is plausible that potential localized suboxic and anoxic microenvironments within the microbial biofilms of *Sargassum* provide ideal conditions for denitrification and nitrification to occur, similar to those seen in seagrass (Brodersen & Kühl, 2022).

Figure 1.10. Presence (filled boxes) and absence (unfilled boxes) of various nitrogen metabolism genes detected in *Sargassum*-associated metagenome-assembled genomes. DNRA and ANR are abbreviations for dissimilatory nitrate reduction to ammonia and assimilatory nitrate reduction, respectively.

Among the possible mechanisms responsible for nitrite production, we hypothesize that the initial step of denitrification or dissimilatory nitrate reduction or nitrification is the likely process contributing to the presence of nitrite in our incubations. If the nitrite observed in our samples is attributable to the activity of denitrifying bacteria capable of performing complete denitrification, then this process could potentially lead to the production of nitrous oxide and dinitrogen, thereby counteracting any source of new nitrogen.

Beyond the nitrogen removal and remineralization process, we uncovered MAGs that have the potential to fix nitrogen and degrade urea. We identified three cyanobacteria of the *Nostocaceae* family that contained the necessary genes to synthesize nitrogenase (*nifD*, *nifK*, *nifH*, and *anfG*; EC.1.18.6.1 or *vnfD*, *vnfK*, *vnfG*, and *vnfH*; EC.1.18.6.2) (Figure 1.10). The cyanobacteria within this family are photosynthetic organisms that are known to be able to fix nitrogen (Bahareh Nowruzi et al., 2021). These cyanobacteria typically occur in colonies made of filaments enclosed in a gelatinous sheath (Bahareh Nowruzi et al., 2021; M. Nicoletti, 2022). Relatives of the Nostoc cyanobacteria, such as *Dichothrix* and *Calothrix*, have been isolated from *Sargassum* tissue samples and have demonstrated the ability to fix nitrogen (Carpenter, 1972; Phlips et al., 1986). Also, MAGs in families *Nostocaceae*, *Elainellaceae*, *Phormidesmiaceae* (phylum *Cyanobacteriota*), *Hyphomonadaceae*, *Rhizobiaceae*, *Stappiaceae*, and *Rhodobacteraceae* (phylum *Pseudomonadota*) encode urease potentially allowing them to utilize urea as a source of nitrogen.

Our findings, in light of previous research, suggest that an active nitrogen cycle associated with *Sargassum* biomes. Since *Sargassum* floats on the surface of the open ocean, atmospheric inputs are likely the most important source of new nutrient for the system. However, the ultimate fate of any nutrient added to the water will depend on the dominant microbial metabolism assimilating the adding nutrients. For instance, if nitrogen cycle extended through to complete denitrification, it could result in *Sargassum* contributing to an overall loss of nitrogen from the Tropical Atlantic Ocean, with the extent of that loss increasing with increased *Sargassum* abundance. We note that we did not determine ammonia or non-*Sargassum* particulate nitrogen in this study, which likely made up a further portion of the lost total oxidized nitrogen.

1.3.9 *Sargassum*-associated bacteria from phyla *Actinomycetota*, *Bacteroidota*, and *Pseudomonadota* degrade aromatic compounds

Pelagic *Sargassum* can release dissolve organic carbon (DOC) in the surrounding seawater at rates ranging from 23 to 41 μg C $g^{-1}_{biomass}$ hr^{-1} (Powers et al., 2019). Recent work shows that polyphenols can account for 5% to 18% of this exuded DOC (Powers et al., 2019). Polyphenols and their derivates are a large group of compounds that are typically characterized by the presence of aromatic rings with hydroxyl groups. The aromatic rings contribute to their stability and resistance to degradation (Belščak-Cvitanović et al., 2018); however, different microbial groups are known to break down polyphenols (Gade & Kumar, 2023; Stevens & Maier, 2016). Considering that pelagic *Sargassum* likely serves as an important source of aromatic polyphenols, particularly in the tropical north Atlantic Ocean, we examined which microbes in our dataset have the metabolic potential to catabolize these compounds.

We detected incomplete pathways responsible for the breakdown of three ring-containing molecules—tris-Cinnamate, phenylacetate, and catechol (Figure 1.11B). The complete catabolism of these compounds results in the production of two central metabolites, acetyl-CoA and succinyl-CoA, which are funneled into the TCA cycle. We found one MAG from the *Alteromonadaceae* family that contained a complete pathway for transforming catechol into succinyl-CoA, and multiple MAGs that encode the catalytic enzymes responsible for the C-ring cleavage (Figure 1.11). The dioxygenase (EC number 1.13.11.16) that cleaves the C-ring during the catabolism of trans-Cinnamate was identified only in the MAGs belonging to phylum *Actinomycetota*. The most abundant catalytic genes involved in cleaving the C-ring are associated with the degradation pathways of phenylacetate and

catechol via meta-cleavage (Figure 1.11A). Majority of the MAGs that may have the metabolic ability to utilize phenylacetate belong to phyla *Actinomycetota*, *Bacteroidota*, *Chloroflexota*, and *Pseudomonadota*. In comparison, only organisms from phyla *Chloroflexota* and *Pseudomonadota* can cleave catechol's aromatic ring. At the family level, majority of the bacteria that employ one or more mechanisms of aromatic compound degradation belong to *Rhodobacteraceae* (phylum *Pseudomonadota*).

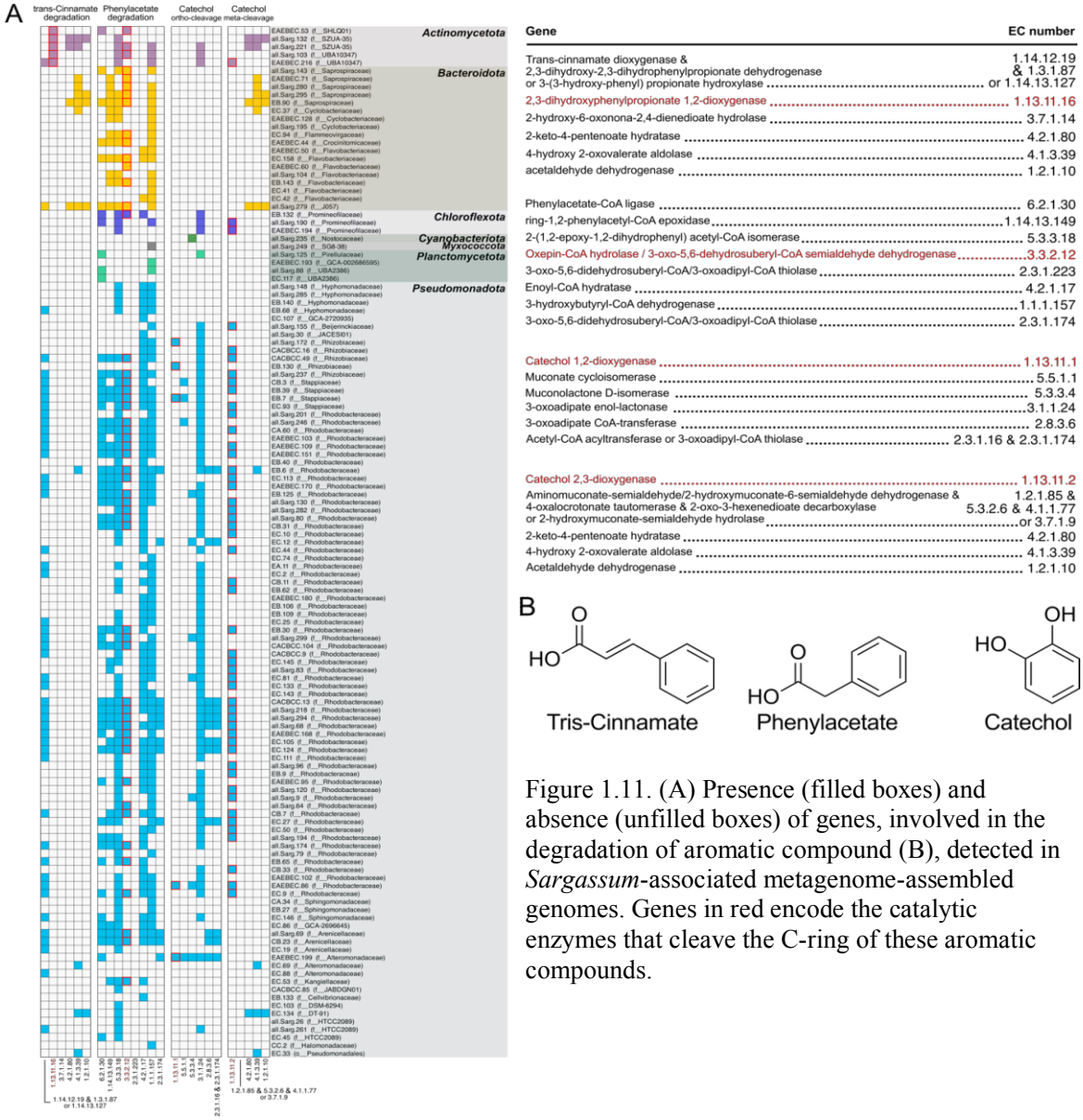


Figure 1.11. (A) Presence (filled boxes) and absence (unfilled boxes) of genes, involved in the degradation of aromatic compound (B), detected in *Sargassum*-associated metagenome-assembled genomes. Genes in red encode the catalytic enzymes that cleave the C-ring of these aromatic compounds.

1.4. Conclusion

This study provides a comprehensive analysis of the microbial communities associated with pelagic *Sargassum* in the tropical north Atlantic Ocean. Our results show that *Sargassum*-associated microorganisms are taxonomically diverse and functionally important in nitrogen cycling and degradation of complex organic compounds. For instance, the presence of nitrogen-fixing cyanobacteria that partner with pelagic *Sargassum* suggests that these organisms may play a significant role in nitrogen acquisition for this macroalga and its microbiota. Moreover, examination of the aromatic compound degradation pathways revealed that *Sargassum*-associated bacteria have the metabolic potential to catabolize carbon ring-containing molecules, thereby contributing to the remineralization of dissolved organic carbon in marine settings. The ability of these microbes to convert complex organic molecules into central metabolites like acetyl-CoA and succinyl-CoA highlights their role in the broader marine carbon cycle.

Overall, our study expands our understanding and knowledge of pelagic *Sargassum's* microbiome. Our findings have significant implications for comprehending the ecological impact of *Sargassum* bloom, which continues to occur annually in the tropical north Atlantic Ocean, extending beyond its historical geographic range (*i.e.*, Sargasso Sea in the western subtropical north Atlantic Ocean). As pelagic *Sargassum* continues to proliferate across the tropical north Atlantic, the functional contributions of its microbes will likely play a crucial role in shaping the nutrient dynamics that could provide insights into the mechanisms behind the bloom.

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Chapter 2 – Siderophores produced by *Sargassum's* microbial symbionts may facilitate iron uptake

Taruna A. Schuelke, Zachary Reitz, Martha Gledhill, Elizabeth Wilbanks

2.1 Introduction

2.1.1 Iron acquisition within the pelagic *Sargassum* holobiont

Iron (Fe) is an essential micronutrient for a variety of metabolic pathways such as photosynthesis, respiration, and nitrogen fixation (Tagliabue et al., 2017). Its importance also extends to the regulation of primary production in some of the world's oceans where iron concentrations are quite low (*e.g.*, tropical eastern Pacific and Southern Ocean) (Boyd et al., 2007). In contrast to these regions, the Atlantic Ocean is generally not considered iron-limited because the Saharan Desert dust clouds fertilize its surface waters with substantial amount of iron throughout the year, especially during summer months (Rodríguez et al., 2021; Sedwick et al., 2005). However, the degree of solubility of this iron, and thus its bioavailability, can vary widely. Furthermore, several processes govern how insoluble iron is transformed into soluble iron (Jickells et al., 2005; Mahowald et al., 2005).

The origin of dust and its composition, atmospheric processing of particles during transport and deposition, and photoreduction of dust upon entry into the seawater have been outlined as key drivers that lead to variation in iron solubility (Baker & Croot, 2010). Beyond these abiotic factors, biological processes, such as grazing, organic complexation of iron bound to ligands, and iron uptake by heterotrophic bacteria, also influence iron solubility in seawater (Baker & Croot, 2010; Breitbarth et al., 2010; Tortell et al., 1996). Solubility of iron directly affects concentrations of dissolved iron that is bioavailable to marine systems.

The pool of dissolved iron is composed of Fe (II), colloidal, soluble, and organically complexed iron (Gledhill & Buck, 2012). Even though determining and characterizing the organic ligands that bind to iron is often difficult, we know that siderophores are indeed constituents of this pool (Gledhill & Buck, 2012). Siderophores are low molecular weight secondary metabolites that are produced by bacteria and fungi to chelate iron (Albelda-Berenguer et al., 2019; J. Chen et al., 2019; Saha et al., 2013). In addition to siderophores, some microorganisms can synthesize other molecules, such as heme, transferrin, and lactoferrin to facilitate iron uptake (Sandy & Butler, 2009), while others rely on Fe^{2+} and Fe^{3+} transporters (Hopkinson & Barbeau, 2012; Toulza et al., 2012). In fact, iron transport proteins are more prevalent in marine bacteria than iron-chelating molecules (Hopkinson & Barbeau, 2012; Toulza et al., 2012). For example, Hogle and colleagues (2015) surveyed 42 Roseobacter and 22 SAR11 genomes from various oceanic regions and found that nearly all organisms contained genes encoding Fe^{2+} and Fe^{3+} transporters. However, only 45% and 40% of the Roseobacters possessed genes allowing them to transport heme- and siderophore-bound iron, respectively, and none of the SAR11 bacteria had genes to encode transporter for ligand-bound iron uptake (Hogle et al., 2016). Moreover, a recent study examining siderophore production and utilization in the North Pacific Ocean found twice as many million reads per liter of seawater for transport genes as for siderophore synthetic genes (Park et al., 2023). Although the importance of all iron-uptake strategies employed by microbes is recognized, this study focuses specifically on siderophores and their biosynthetic gene clusters.

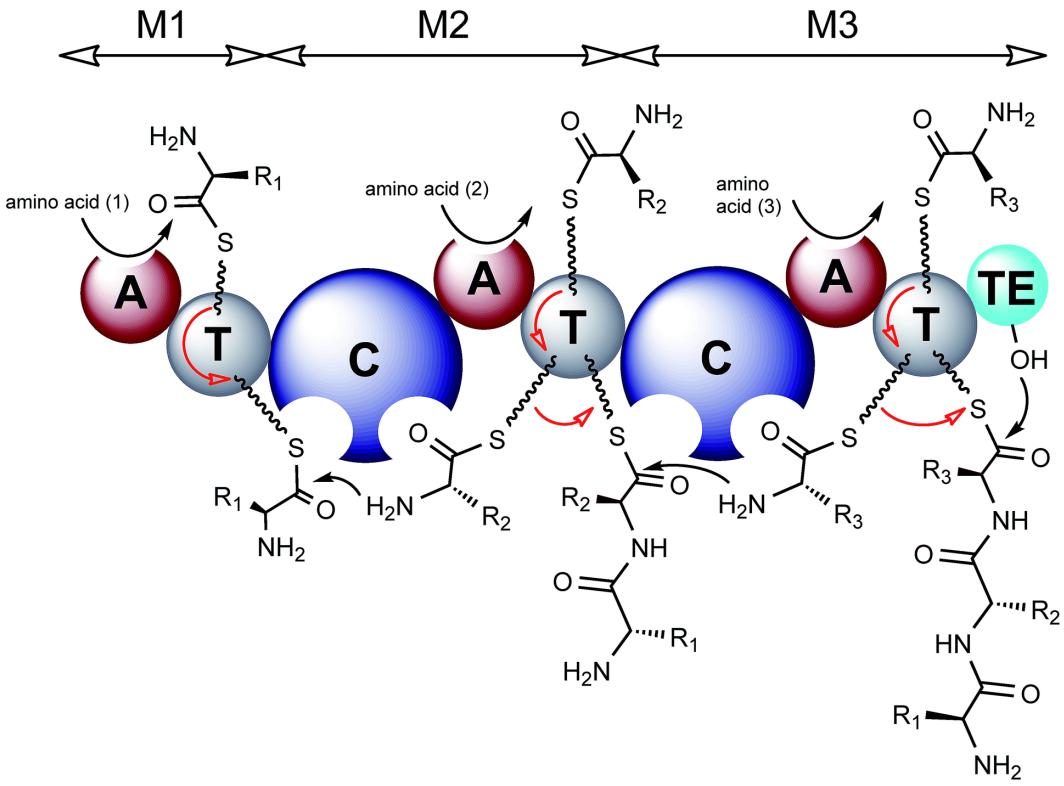
The primary research goal of this part of the investigation is to explore whether microbial associates of pelagic *Sargassum* produce siderophores, and if so, to characterize

the siderophores and the biosynthetic pathways the microbes use to produce them. To achieve this goal, we utilized a combination of biogeochemical and metagenomic data to characterize siderophores produced by *Sargassum's* microbial partners and to identify the biosynthetic pathways of these iron-chelating molecules, respectively. The following introductory section provides an overview of these biosynthetic pathways.

2.1.2 Siderophore biosynthetic pathways

Siderophores are produced via two pathways–**the non-ribosomal peptide synthetase (NRPS) pathway** and the **NRPS-independent siderophore (NIS) synthetase pathway** (Carroll & Moore, 2018). These two pathways differ from the typical protein synthesis process during which amino acids are joined together by the ribosomes based on the information transcribed in the message RNA (mRNA). In contrast, NRPSs are enzymatic assembly lines comprised of multiple modules. A typical NRPS module is responsible for adding a single amino acid to the peptide chain, and it is composed of three domains with distinct functions: an adenylation (A) domain, a condensation (C) domain, and a peptidyl carrier protein (PCP) domain. The PCP domain is also known as the thiolation (T) domain. The A domain activates a very specific monomer via adenylation and loads the activated substrate onto the PCP/T domain. The intermediate compounds are tethered to the PCP/T domains which transfers the bound substrate to and from the condensation domains. The C domain catalyzes the peptide bond between the amino acids of the upstream and downstream PCP/T domains, resulting in the transfer of the amino acid or peptide onto the substrate of the downstream PCP/T domain (Figure 2.1) (Gulick, 2017; Miller & Gulick, 2016). Beyond these core domains, an NRPS can contain epimerization, heterocyclization, reduction, oxidation, and N-methylation domains (Fischbach & Walsh, 2006).

Due to the conserved structure of NRPS domains, and the assembly-line mode of peptide synthesis, the number and type of monomers used to form these peptides may easily be predicted (Carroll & Moore, 2018). However, this seemingly straightforward method of predicting the siderophore product is complicated by additional chemical transformations that the C domains may perform or in cases where the domain architectures deviate from the typical structure. Nevertheless, it is known that almost all siderophores produced by NRPSs incorporate one or more of a limited number of chelating moieties (Figure 2.2A). The genes responsible for the production of these moieties are known and can be leveraged as biomarkers to detect the biosynthetic gene clusters of NRPS siderophores (Reitz et al., 2022). These genes, their precursors, products, and example siderophores are illustrated in figure 2.2.



In addition to synthesis by NRPSs, several other siderophores are **NRPS-independent siderophores** (**NISs**) which are produced by NIS synthetases. These

synthetases perform a single enzymatic reaction between a carboxylate and an amine or an alcohol group, and they are divided into three main class types based on substrate specificity. Type A synthetases have specificity for citrate. Type B enzymes utilize α-ketoglutarate and citryl-amine intermediate substrates. Finally, type C enzymes bind to citryl- or succinyl-based intermediates (Carroll & Moore, 2018). All NIS synthetases studied to date have conserved N-terminal IucA/IucC catalytic domain and a conserved C-terminal domain homologous to FhuF iron transporter proteins (Carroll & Moore, 2018).

Aerobactin was the first siderophore known to be produced by an NIS synthetase (Challis, 2005). The genes, their products, and their sequences of the aerobactin biosynthetic pathway were first characterized from a large plasmid, pCoIV-K30, in *E. coli* (Carbonetti & Williams, 1984; De Lorenzo et al., 1986; Martìnez et al., 1994; Warner et al., 1981). The aerobactin operon spans a region of approximately 8 kilobases (kb) within this plasmid, and it contains five genes—*iucABCD* and *iutA* (Carbonetti & Williams, 1984; De Lorenzo et al., 1986). The former set of genes encode biosynthetic enzymes, and the latter gene produces a membrane receptor protein.

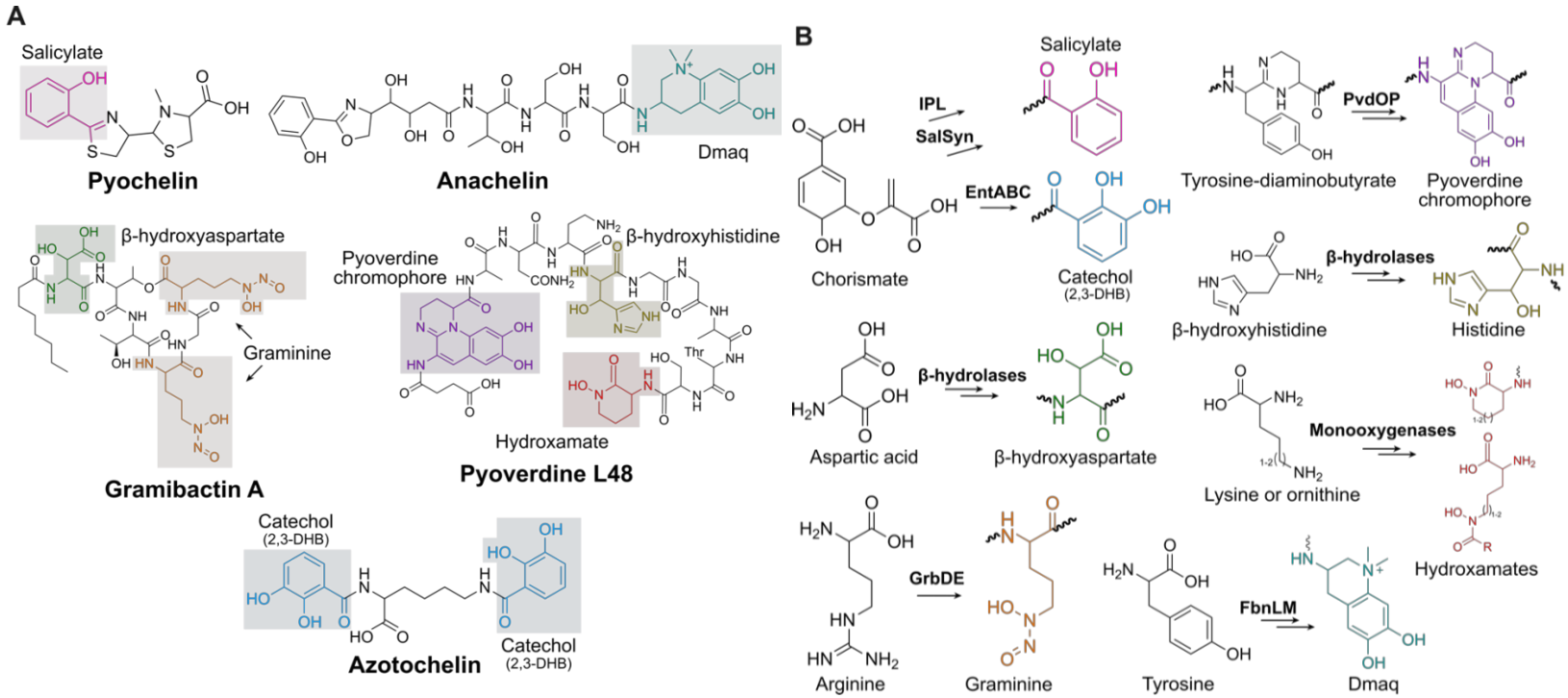


Figure 2.2. (A) Commonly found iron chelating moieties shown in representative bacterial siderophores made by NRPSs. The substructures containing the chelating moieties are shaded in different colors. (B) The biosynthetic pathways of these substructures were reproduced from Reitz et al., 2022 with permission. The substructures are shown in colors which correspond to the shaded regions in part A. 2,3-DHB is 2,3-dihydroxybenzoate, and dmaq is the short form of 1,1-dimethyl-3-amino-1,2,3,4-tetrahydro-7,8-dihydroxy-quinoline. Gene names and products are as listed—*IPL*: isochorismate pyruvate-lyase; *SalSyn*: bifunctional salicylate synthase; *EntABC*: 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase, isochorismatase, and isochorismate synthase, respectively; *PvdOP*: oxidoreductase and tyrosinase, respectively. *GrbDE*: graminine synthesis protein and arginine hydroxylase, respectively; *FbnLM*: Dmaq synthesis proteins.

2.2 Methods

2.2.1 Sampling and incubating Sargassum

Six *Sargassum* samples were collected from the sea surface aboard the RV *Meteor* on April 27, 2018, during the M147 GEOTRACES process cruise (Gapr11; 4°9'0" N 42°53'60" W). Three of these samples were frozen at -80°C immediately upon collection. These tissue samples are designated as CA, CB, and CC where C stands for control and A-C are biological replicates The other three tissue samples were used for onboard incubations with the goal of assessing which metabolites, namely siderophores, do microorganisms, associated with *Sargassum*, are able to produce. The experimentally incubated samples are EA, EB, and EC where E stands for experimental and A-C are biological replicates.

Seawater from the same location as listed above was collected at a 500 m depth, and it was 0.22-μm filtered to be used for incubating the *Sargassum* tissue. We used nutrient-rich, deep-sea water to alleviate macronutrient limitation. Six trace-metal clean 2L polycarbonate bottles were filled with 2L of the 0.22-μm filtered deep seawater. *Sargassum* tissue was placed in three experimental bottles (*i.e.*, Eaw, Ebw, Ecw), and the other three bottles were designated negative controls containing no tissue (*i.e.*, Caw, CBw, CCw). All six bottles were incubated in a deckboard incubator for 24 hours. Tissue samples were collected before and after the incubation period for elemental analysis and molecular work. Seawater samples were collected before and after the incubation period for measuring macronutrients and dissolved organic carbon (DOC). We presented the tissue elemental data, seawater macronutrients and DOC data in chapter 1. The incubated water samples from all six bottles were also filtered using a 0.22-μm polyvinylidene fluoride membrane filter (Sterivex-GV, Millipore) and then passed through a 200-mg modified polystyrene divinyl benzene SPE

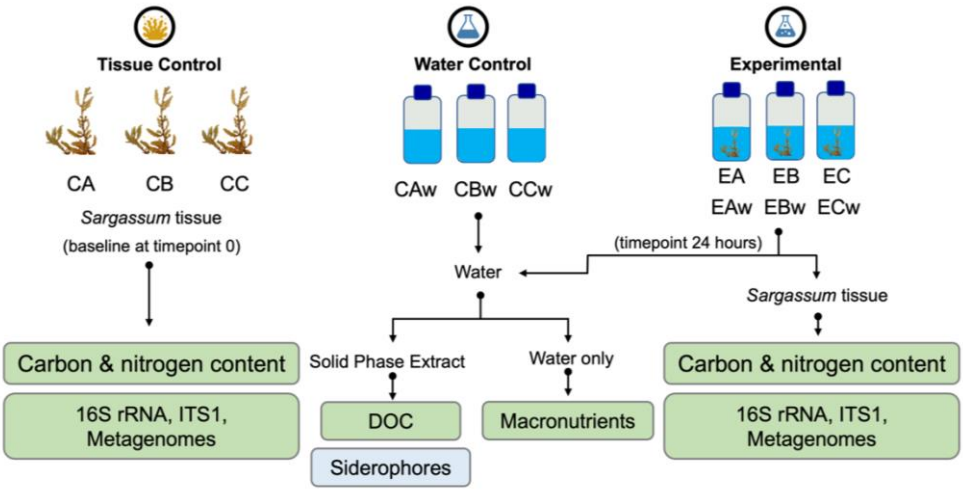


Figure 2.3. An overview of the experimental design implemented during the M147 GEOTRACES process cruise in April 2018. The data types in green and blue boxes are discussed chapter 1 and this chapter, respectively.

column cartridge (ENV+, Biotage, Sweden) at ambient pH. Extractions were carried out at room temperature in the dark. A vacuum manifold was used at a flow rate of ~10 mL min^{-1} during filtration and solid phase extraction processes (Gledhill, Hollister, et al., 2022). The Sterivex-GV and column cartridges were dried and frozen at -20°C for future processing at GEOMAR. The Sterivex-GV filters and the ENV+ columns were used to detect particulate and dissolved metabolites, respectively. An overview of the experimental setup is presented in figure 2.3.

2.2.2 Identifying siderophores produced by *Sargassum's* microbiota

High purity water (18.2 mΩcm^{-1}, Milli-Q, Millipore), and LC-MS grade solvents and ammonium formate (Fisher) were used throughout. Solid phase extraction columns were washed with 5 mL 10 mmol L^{-1} ammonium carbonate to remove inorganic salts. Siderophores were eluted with 5 mL 81:14:5:1 (vol:vol:vol:vol) acetonitrile: propan-2-ol: H_2O: formic acid (Gledhill, Hollister et al., 2022). 1 mL aliquots were reduced to ~ 100 µL in a centrifugal evaporator and either analyzed directly or after addition of excess Ga. Ga was added to samples to facilitate siderophore identification via metal isotope fingerprinting in

Chelomex (Baars et al., 2014) following the approach described previously (Gledhill, Zhu, et al., 2022). Samples (25 µL) were analyzed by high performance liquid chromatography (HPLC, Biocompatible Ultimate 3000) coupled in parallel to a high-resolution electrospray ionization mass spectrometer (ESI-MS, Orbitrap Q Exactive, Thermo) and a magnetic sector inductively coupled plasma mass spectrometer (ICP-MS, Element XR, Thermo). The fraction of eluate going to the ICP-MS detector was dried with a desolvator (Aridus II, CETAC) and oxygen added to nebulizer gas at a flow rate of 20 mL min^{-1} to prevent the build-up of carbon on the platinum skimmer cones of the ICP-MS (Boiteau et al., 2013). The ^{56}Fe intensity was monitored for siderophore detection. Siderophores were separated using a Hamilton PRP C18 5 µm 150 × 2.1 mm PEEK column using a gradient from 5:95 methanol: 10 mM ammonium acetate (pH 6.7, vol:vol) to 100 % methanol over 15.5 minutes at a flow rate of 400 µL min^{-1} followed by 2.5 min at 100 % methanol prior to returning to the start conditions for equilibration (5 mins). Chromatograms from the ESI-MS and ICP-MS were aligned via analysis of a standard solution of ferrioxamine B.

2.2.3 Identifying gene clusters responsible for biosynthesizing siderophores

Using antiSMASH 7.0, we annotated and analyzed the secondary metabolite biosynthetic gene clusters (BGCs) in our dereplicated metagenomic assembled genomes (MAGs) described in chapter 1 (Blin et al., 2023). In case we were not able to locate the BGCs of interest, we used the unbinned metagenomic co-assembly that was built using reads from all our samples to search for these gene clusters. We did this by matching contigs and by mapping raw reads to reference BGCs from organisms known to produce the siderophores in question. We used BLAST v2.9 and Bowtie 2 v2.4.4 to match contig and to map reads, respectively (Camacho et al., 2009; Langmead & Salzberg, 2012). We downloaded the

reference sequences from either previously published work or we built our own set of references. In case of the latter, we built draft HMMs using the protein sequences of the monooxygenase enzyme that synthesizes the iron-chelating moiety (*i.e.*, hydroxamate in our case) of the siderophores under investigation. The HHMs were iteratively refined by adding putative monooxygenase-encoding sequences to the model that were manually confirmed. Next, we created a rule within a local version of antiSMASH 7.0 in order to parse out NRPSs that form hydroxamate siderophores.

2.3 Results and discussion

2.3.1 Siderophore identification

We identified a total of 11 siderophores in our solid phase extracts that were purified from the final, experimental seawater samples after a 24-hour incubation period (Table 2.1 & Figure 2.4). All 11 siderophores use the hydroxamate binding moiety and belong to three types of classes—ferrioxamines, amphibactins, and ferrichromes. The four types of ferrioxamines that we detected in our samples were ferrioxamine A1, B, E, and G. Ferrioxamines are the iron-bound forms of desferrioxamines (DFO), and we use the two terms interchangeably. Amphibactins are cell-associated, amphiphilic peptide siderophores that were first isolated from a marine Gram-negative bacterium, *Vibrio* sp. R-10 (J. S. Martinez et al., 2003). The headgroup of amphibactins is comprised of four amino acids—one L-serine, one L-ornithine, and two D-ornithines (Figure 2.4). The ornithine residues are hydroxylated and acetylated to create the hydroxamate units that chelate the iron (III) (J. S. Martinez et al., 2003). The fatty acid chain is ligated to the N2 of L-ornithine, and these chains can vary in their degree of unsaturation and hydroxylation (J. S. Martinez et al., 2003). We uncovered five specific amphibactins in our samples—amphibactins D, B, E, C, and F.

The two fungal siderophores that were found in our incubated samples were ferrichrome and ferricrocin. Both compounds belong to a large family of siderophores called ferrichromes. Table 2.1 lists the identities, masses, and the retention times of the siderophores we identified.

Identity	[M-2H+^{56}Fe]$^{+}$	Retent. Time ($NH_4CH_3CO_2$)	Retent. Time (NH_4HCO_2)	Retent. Time ($NH_4CH_3CO_2$)	Retent. Time (NH_4HCO_2)
Ferrioxamine A1	600.2566	n.d.	8.41	4	7.9
Ferrioxamine B	614.2724	4.28	8.82	4.1	8.3
Ferrioxamine E	654.267	n.d.	9.09	4.79	9.1
Ferrioxamine G	672.2779	3.52	6.25	3.44	6
Ferrichrome	741.2379	4.33	8.21	4	7.37
Ferricrocin	771.248	n.d.	8.26	n.d.	7.02
Amphibactin D	885.4141	8.98	14.4	8.78	14.3
Amphibactin B	901.409	8.34	13.71	8.17	13.6
Amphibactin E	911.429	9.3	14.8	9.11	14.7
Amphibactin C	927.4246	8.7	14.21	8.54	14.1
Amphibactin F	929.4403	9.21	14.72	9.06	14.62

Table 2.1. Identities, masses, and retention times of the siderophores detected in the solid phase extracts from the incubated bottles containing the *Sargassum* tissue.

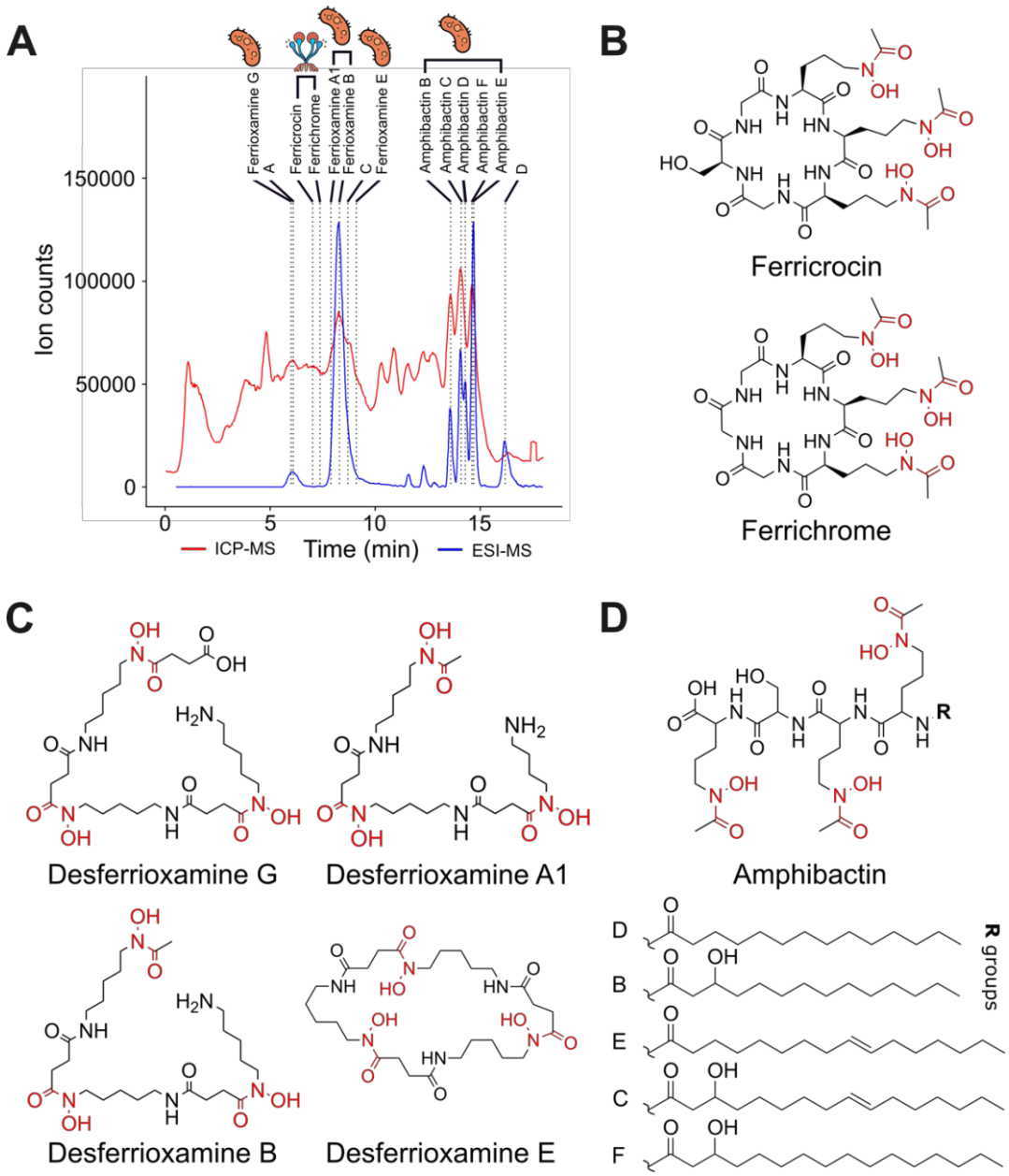


Figure 2.4. (A) Representative chromatograms of samples collected from the incubated bottles (T = 24 hrs.) containing *Sargassum* samples. The red and blue lines are for data from ICP-MS and ESI-MS, respectively. indicates bacterial siderophores and indicates fungal siderophores. In addition to identifying siderophores based on references, we detected three unknown compounds. (B) Chemical structures of ferricrocin and ferrichrome. (C) Chemical structures of the four ferrioxamines we detected in our samples. The structures are of the unbound iron ferrioxamines—desferrioxamines. (D) Chemical structures of five amphibactins we identified in our samples. The core structure of these amphibactins is presented along with their R groups. (B-D) The hydroxamate moieties are shown in red.

Hydroxamate siderophores, along with catecholate, are common siderophores produced by microorganisms, including marine bacteria and fungi (Reitz et al., 2022, Figure 2) . Previous investigations, conducted across diverse marine systems and involving cultured relatives of marine bacteria, have uncovered multiple hydroxamate siderophores (Gledhill et al., 2004; Granger & Price, 1999; J. S. Martinez et al., 2000; Mawji et al., 2008, 2011; Park et al., 2023; Trick, 1989; I. Velasquez et al., 2011; I. B. Velasquez et al., 2016). Therefore, the presence of hydroxamate siderophores in our samples is not surprising.

Desferrioxamines and amphibactins are synthetized by bacteria and were detected in all of our biological replicates, EA, EB, and EC. Desferrioxamines and amphibactins have been detected in various oceanic regions around the globe. One of the first studies on siderophores in the seawater revealed that ferrioxamine E was a ubiquitous iron chelator in the water and sediment samples collected from the Baltic Sea (Kosakowska et al., 1999). Furthermore, a few prominent studies reported that siderophores of the ferrioxamine and amphibactin family are prevalent in the surface waters of the Atlantic Ocean, which is the same region as this investigation (Gledhill et al., 2004; Gledhill, Zhu, et al., 2022; Mawji et al., 2008, 2011). Beyond the Atlantic, ferrioxamine and amphibactin siderophore-types have been found in other regions including the North Pacific and the South Pacific Oceans (Boiteau et al., 2016, 2019; Park et al., 2023; I. Velasquez et al., 2011; I. B. Velasquez et al., 2016).

In addition to ferrioxamines and amphibactins, we identified two ferrichrome siderophores which are fungal in origin (Hider & Kong, 2010). Research reports on siderophores produced by marine fungi are limited. However, a few studies have found that marine fungi are indeed capable of synthesizing hydroxamate, catecholate, and carboxylate

siderophores (Baakza et al., 2004; Huang et al., 2020; Park et al., 2023; Vala et al., 2006) . To the best of our ability, we found no reports of marine fungi that produce ferrichrome siderophores, which suggests that our study is the first to identify such activity.

After identifying the siderophores produced by the microbial associates of pelagic *Sargassum*, we surveyed the MAGs of these microbes to find the biosynthetic gene clusters (BGCs) that encode enzymes for manufacturing these siderophores. In addition to identifying microorganisms that are potentially responsible for biosynthesizing amphibactins, ferrioxamines, and ferrichromes, we search our MAGs for BGCs of other siderophores. We discuss our findings in the subsequent subsections starting with the two bacterial siderophores (*i.e.*, ferrioxamines and amphibactins) followed by fungal siderophores from the ferrichrome family.

2.3.2.a Ferrioxamine (NIS)

Desferrioxamines are nonpeptide hydroxamate siderophores that are synthesized by the NRPS-independent siderophore (NIS) synthetase pathways (Carroll & Moore, 2018; Challis, 2005). The biosynthetic pathway of these siderophores was first identified in *Streptomyces pilosus* (Schupp et al., 1987, 1988) and *Streptomyces coelicolor* M145 (Barona-Gómez et al., 2004). An overview of the gene cluster and the biosynthetic pathway is presented in figure 2.5. Briefly, the first step in this biosynthetic pathway is the decarboxylation of L-lysine by desA, a pyridoxal phosphate (PLP)-dependent lysine decarboxylase. The decarboxylation step results in the formation of diamine substrate 1,5-diaminopentane (DP, also known as cadaverine) (Schupp et al., 1987, 1988). Next, DP is mono-*N*-hydroxylated by desB, an FAD-dependent monooxygenase resulting in *N*-hydroxy-1,5-diaminopentane (HDP) (Schupp et al., 1988). In the third step, desC, an acyl CoA-dependent acyl transferase, *N*-acetylates

or *N*-succinylates HDP to produce *N*-acetyl-*N*-hydroxy-1,5-diaminopentane (AHDP) or *N*-succinyl-*N*-hydroxy-1,5-diaminopentane (SHDP), respectively (Ronan et al., 2018). The last step involves the condensation of one AHDP molecule to two SHDP molecules to form desferrioxamine B or the condensation of three SHDP molecules to form desferrioxamine E. This last step is catalyzed by desD, an NTP-dependent synthetase (Telfer et al., 2019).

We detected only one MAG (*i.e.*, EB.133, Chapter 1: Table 1.6) containing the biosynthetic genes, DesA-D, responsible for making ferrioxamine. EB.133 was 96% complete with an N50 of 17.8 kb, and it was identified as a member of the *Cellvibrionaceae* family (phylum *Pseudomonadota*, class *Gammaproteobacteria*). Based on

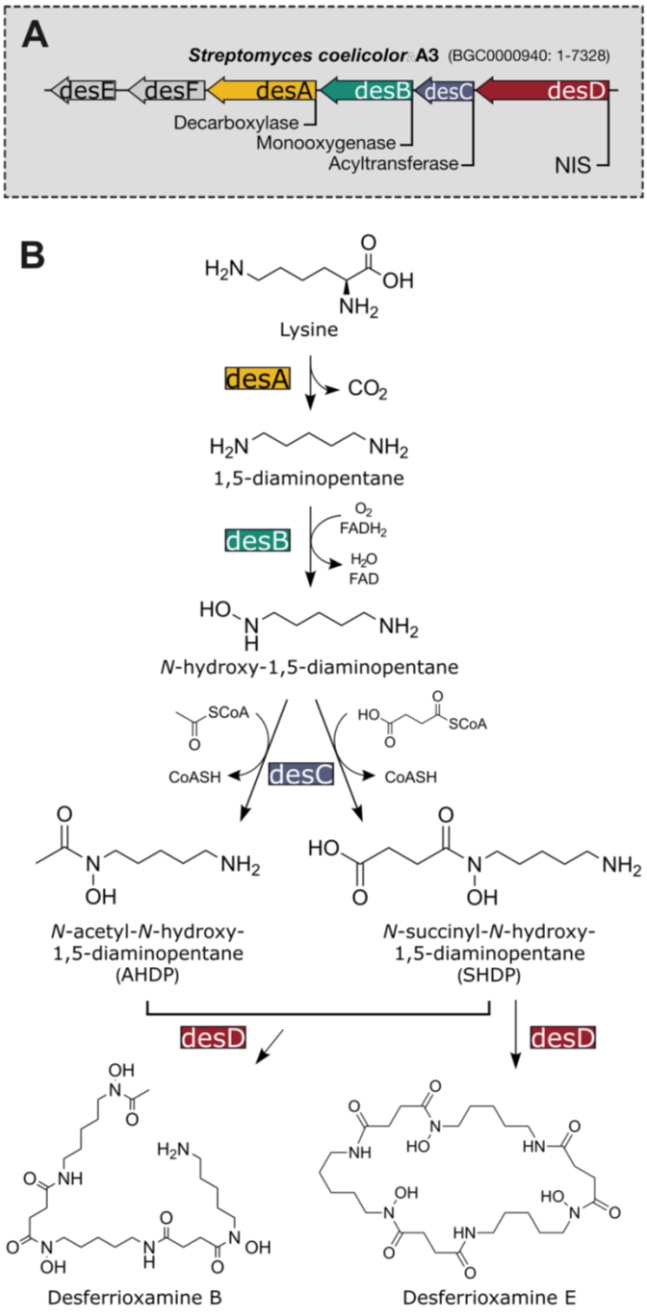


Figure 2.5. (A) The desferrioxamine biosynthetic gene cluster from *Streptomyces coelicolor* A3. DesE and desF respectively encode for a receptor protein and a putative reductase. (B) the biosynthetic pathway from the same bacterium as the gene cluster.

our 16S rRNA data presented in chapter 1, *Cellvibrionaceae* family accounted for 4.80% and 11.76% of the bacterial community in our initial and final samples, respectively (Chapter 1: Figure 1.4). This finding confirms taxonomic consistencies between our amplicon and the metagenomic dataset. Uncovering a BGC in only one MAG that potentially encodes for several types of desferrioxamines is not an unprecedented finding. For instance, Senges et al. 2018 identified a single ferrioxamines synthesis gene cluster in the genome of *Streptomyces chartreusis* despite the fact that this bacterium is capable of producing a suite of ferrioxamines depending on the growth conditions (Senges et al., 2018).

The closest blast matches to the entire gene cluster of our MAG were members of the same class as the MAG—*Gammaproteobacteria* (Figure 2.6). Although desferrioxamines are mainly produced by members of the *Actinomycetia* class, other unrelated bacteria belonging to classes *Gammaproteobacteria* and *Alphaproteobacteria* are also known to make siderophores in this family (Essén et al., 2007; Park et al., 2023; Smits & Duffy, 2011). Therefore, it is expected that the closest matches to our single MAG are also from the class *Gammaproteobacteria*.

2.3.2.b Amphibactin (NRPS)

We uncovered five specific amphibactins in our samples—amphibactins D, B, E, C, and F (Table 2.1 and Figure 2.4). As mentioned previously, the headgroup of amphibactins is comprised of four amino acids—one L-serine, one L-ornithine, and two D-ornithines (Figure 2.4). The fatty acid chain is ligated to the N2 of L-ornithine, and these chains can vary in their degree of unsaturation and hydroxylation (J. S. Martinez et al., 2003). Amphibactins are cell-associated, amphiphilic peptide siderophores that were first isolated from a marine Gram-negative bacterium, *Vibrio* sp. R-10 (J. S. Martinez et al., 2003). It has been suggested

that the cell association potentially guards against loss of the siderophore via diffusion in the aquatic environment (J. S. Martinez et al., 2003).

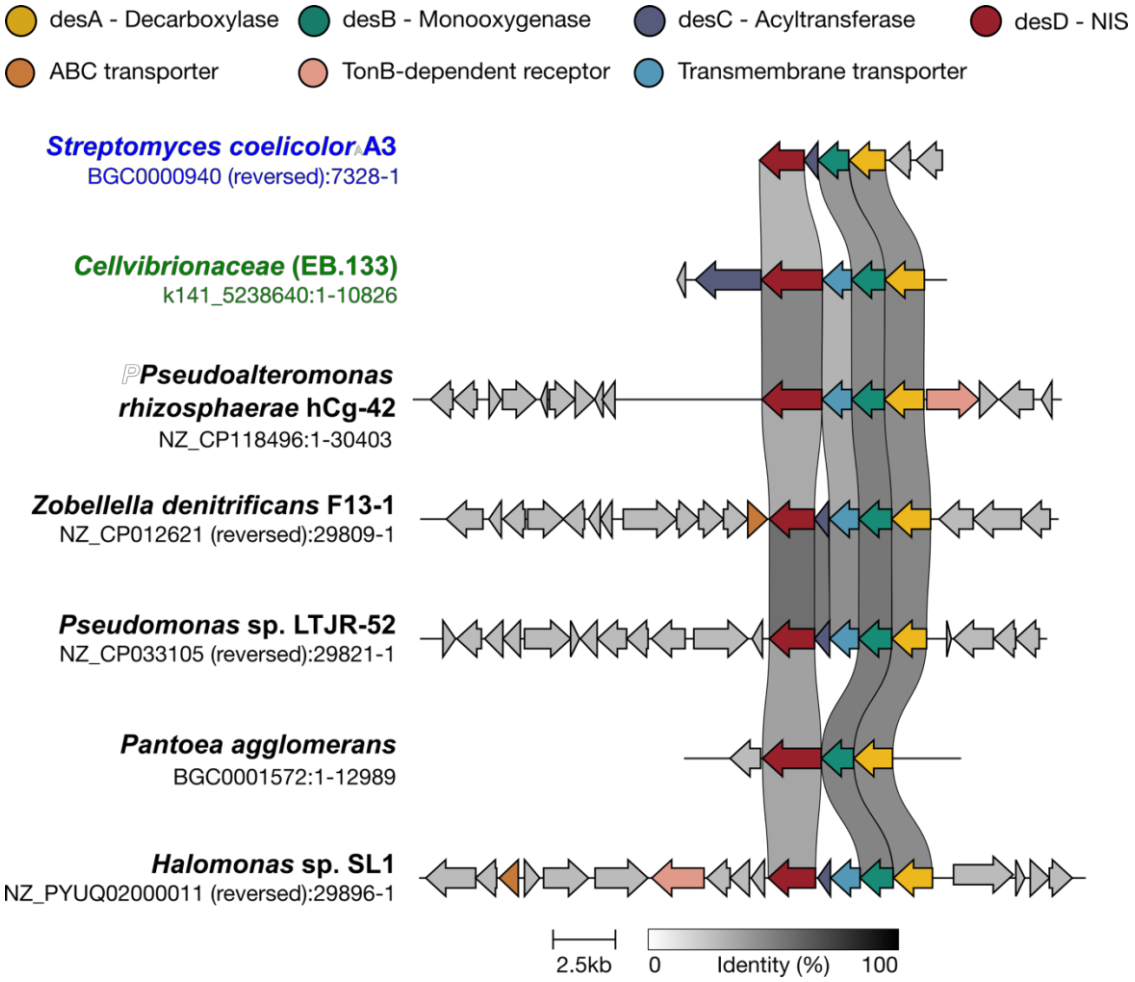


Figure 2.6. Homology among the desferrioxamines biosynthetic gene clusters of various members of the *Pseudomonadota* phylum, class *Gammaproteobacteria*, that matched to our MAG (green) which also belongs to the same phylum. The desferrioxamine BGC was first identified in *Streptomyces coelicolor* A3 (blue).

Amphibactins are synthesized by the NRPS pathway (Kem et al., 2014). The biosynthetic gene cluster responsible for the production of amphibactins was initially identified in an oil-degrading marine bacterium, *Alcanivorax borkumensis* SK2 (Kem et al., 2014). Sequence-based comparisons revealed that two genes, ABO_2093 and ABO_2092, make up a putative NRPS. This potential NRPS is upstream of other genes that are known to be involved in the formation of ornithine-derived siderophores. Figure 2.7 depicts the biosynthetic gene cluster along with NRPS domain architecture and gene functions in *A. borkumensis SK2* (accession no. NC_008260).

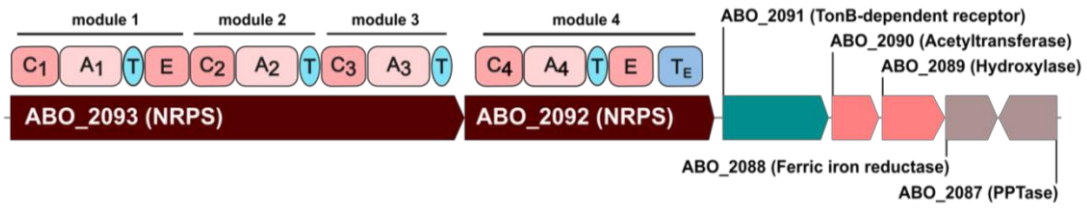


Figure 2.7. The amphibactin biosynthetic gene cluster and the domain architecture in *Alcanivorax borkumensis* SK2 (accession no. NC_008260).

We did not detect any MAGs containing an NRPS that is responsible for synthesizing amphibactin. This is likely due to the fragmented nature of the MAGs, a challenge frequently encountered by researchers working with metagenomic data. Furthermore, NRPS encoding biosynthetic gene clusters are significantly larger than NIS clusters, complicating their identification in MAGs even further. As a result, we compared the reads and a co-assembly from all six samples to a curated set of 20 reference amphibactin BGCs. More information on these references is provided in Appendix § II. These references were extracted from networks built based on sequence similarity shared by NRPS gene clusters (Reitz et al., 2022). Of these reference clusters, we were able to match contigs and reads to the NRPS regions of only seven species, and all of these species belong to the genus *Vibrio* (Figure 2.8). These species are either related to or are known pathogens of marine mollusca and corals ((Dubert,

Romalde, Prado, et al., 2016; Dubert, Romalde, Spinard, et al., 2016; Galvis et al., 2021; Lasa et al., 2021; Ushijima et al., 2020; Yilmaz et al., 2023). Among the aforementioned *Vibrio* species to which our co-assembled contigs and reads matched, three of the species have been found to be associates of pelagic *Sargassum—V. europaeus*, *V. tubiashii*, and *V. corallillyticus* (Michotey et al., 2020; Theirlynck et al., 2023). Furthermore, we also detected the best matched reference species, *Vibrio gigantus*, in our ASVs, albeit at a very low abundance. Our results show that some members of the *Sargassum* microbiome have the genetic capacity for amphibactin biosynthesis, using enzymes closely related to those described in cultivated *Vibrio* species. Due to the fragmented nature of these sequences, the taxonomic identity of the organism encoding these genes for amphibactin biosynthesis remains unclear.

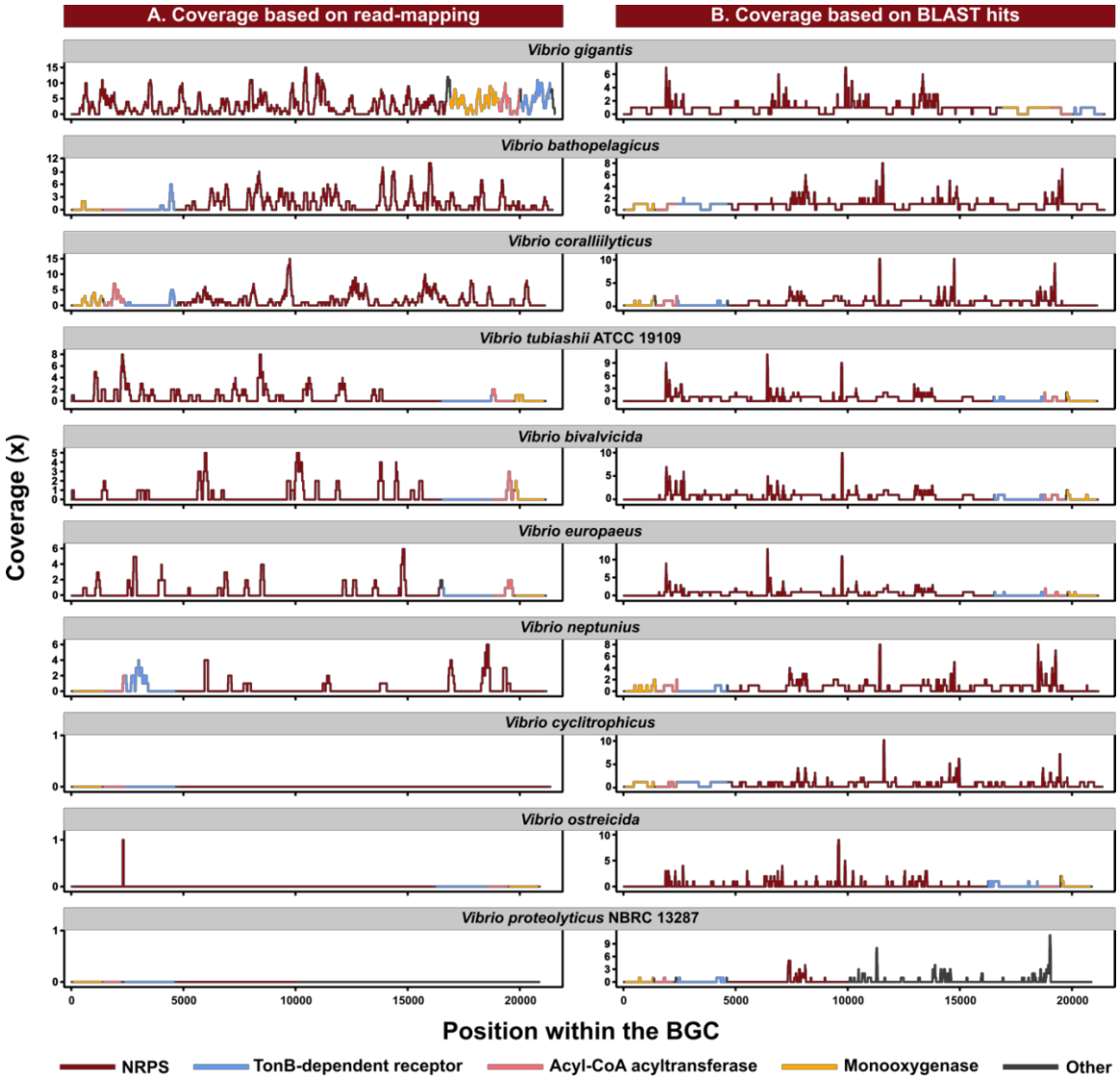


Figure 2.8. (A) Read- and (B) BLAST-based plots showing coverage per base within the 20 reference gene clusters (BGCs) responsible for synthesizing amphibactin. Each base position within the BCGs is color-coded based on putative functions of the encoded enzymes.

2.3.2.c Ferrichrome and ferricrocin (NRPS)

Although the genes responsible for ferrichrome and ferricrocin biosynthesis have been characterized in cultivated terrestrial fungi, we faced several challenges in identifying the BGCs for these fungal siderophores in our metagenomic data. Consequently, we employed an approach analogous to the one used to uncover the amphibactin-producing BGCs in our search for the gene clusters associated with our fungal siderophores. However, unlike the amphibactin reference BGCs, a very small number of ferrichrome-producing NRPSs have been described in fungi. To remedy this, we started by building a draft HMM using the protein sequences of the monooxygenase enzyme that synthesizes the iron-chelating hydroxamate moiety of the ferrichrome family siderophores. This initial HMM was constructed using sequences from the six known examples of NRPSs that manufacture siderophores classified as ferrichrome-types (Jenner et al., 2023 and references therein). The HHM was iteratively refined by adding putative monooxygenase-encoding sequences to the model that were manually confirmed. Our final HMM was created using 15 sequences listed in Table 2.2. Next, we created a rule within a local version of antiSMASH 7.0 in order to parse out fungal NRPSs that form hydroxamate siderophores. We executed this search against the fungal sequences in the antiSMASH database. We clustered our hits into networks based on sequence similarity among the NRPSs and manually selected references that had the metabolic potential to synthesize ferrichrome-type siderophores. Identifying references using networks was an important step in our pipeline because domain architectures in fungi for producing ferrichrome-type siderophores varies from species to species (Bushley et al., 2008). Moreover, during our research, we discovered that the monooxygenase gene is not always located near the NRPS in some fungi such as *Aspergillus*.

Hence, network-based filtering was necessary. After obtaining a set of reference NRPSs, we conducted a BLAST search using the contigs from our unbinned co-assembly, which was constructed with read pairs from all samples. We mapped the reads to the references but no contigs and reads matched to the fungal references.

This finding could be explained by the lack of fungal reads in our dataset due to their low abundance of fungi, or, more plausibly, it reflects the significant divergence of fungal species in our samples from the references in the current databases. For instance, an assessment of the widely used reference databases, such as NCBI-nr/nt and UNITE, concluded that only about 35% to 41% of the queried ITS amplicons from marine habitats could be classified, and even then, only at the phylum level (Khomich et al., 2018). Our findings reflect this limitation of the reference databases. Specifically, 61% and 16% of our ITS1 ASVs were unclassified or had uncertain placement in the fungal phylogeny (*i.e.*, *Incertae sedis*), respectively (Figure 2.9). This underscores the critical need for an improved characterization of marine fungal diversity, including the development of comprehensive reference genomes and cultures.

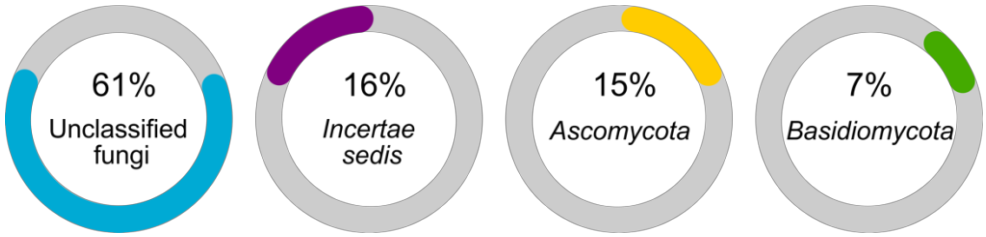


Figure 2.9. Percentage of ITS1 amplicon sequence variants that were unclassified or classified as members of the phyla *Ascomycota* or *Basidiomycota*.

Despite not detecting the fungal BGCs, we postulate that species in the *Aspergillaceae* family may be responsible for the production of ferrichrome and ferricrocin identified in our incubated samples using our mass spectrometry data. Our rationale for this

conjecture is based on our ITS1 results in which the relative abundance of *Aspergillaceae* ASVs increased from 0.714% in the control samples to 93.3% in the incubated experimental samples (Mann-Whitney U test, p-value=0.0005). Particularly, ASVs from the genera *Aspergillus* and *Penicillium* accounted for 1.12% and 60.31% of the total ASVs in the incubated samples (Chapter 1: Figure 1.5). These outcomes are noteworthy because terrestrial species of these genera are known to produce ferrichrome and ferricrocin (Charlang et al., 1981; Eisendle et al., 2003; Wallner et al., 2009).

Accession ID	Species	Ref PubMed ID	Phylum	Class	Order	Family	Genus	Species
AY223811.1	*Aspergillus nidulans*	12828635	*Ascomycota*	*Dothideomycetes*	*Eurotiales*	*Aspergillaceae*	*Aspergillus*	*nidulans*
N4WYI1.1	*Bipolaris maydis* ATCC 48331	23236275	*Ascomycota*	*Dothideomycetes*	*Pleosporales*	*Pleosporaceae*	*Bipolaris*	*maydis*
OBU00456.1	*Pseudogymnoascus verrucosus*	unpublished	*Ascomycota*	*Leotiomycetes*	*Thelebolales*	*Thelebolaceae*	*Pseudogymnoascus*	*verrucosus*
Q9P7T0.1	*Schizosaccharomyces pombe* 972h-	11859360	*Ascomycota*	*Schizosaccharomycetes*	*Schizosaccharomycetales*	*Schizosaccharomycetaceae*	*Schizosaccharomyces*	*pombe*
I1RN13.1	*Fusarium graminearum* PH-1	17823352	*Ascomycota*	*Sordariomycetes*	*Hypocreales*	*Nectriaceae*	*Fusarium*	*graminearum*
A8NF99.1	*Coprinopsis cinerea* okayama7#130	20547848	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Psathyrellaceae*	*Coprinopsis*	*cinerea*
TFL07489.1	*Pterula gracilis*	30886374	*Basidiomycota*	*Agaricomycetes*	*Agaricales*	*Pterulaceae*	*Pterula*	*gracilis*
EJD35976.1	*Auricularia subglabra* TFB-10046 SS5	unpublished	*Basidiomycota*	*Agaricomycetes*	*Auriculariales*	*Auriculariaceae*	*Auricularia*	*subglabra*
KZV86816.1	*Exidia glandulosa* HHB12029	26659563	*Basidiomycota*	*Agaricomycetes*	*Auriculariales*	*Exidiaceae*	*Exidia*	*glandulosa*
KIO13719.1	*Pisolithus tinctorius* Marx 270	unpublished	*Basidiomycota*	*Agaricomycetes*	*Boletales*	*Pisolithaceae*	*Pisolithus*	*tinctorius*
EIN04584.1	*Punctularia strigosozonata* HHB-11173 SS5	unpublished	*Basidiomycota*	*Agaricomycetes*	*Corticiales*	*Punctulariaceae*	*Punctularia*	*strigosozonata*
TDL27311.1	*Rickenella mellea*	unpublished	*Basidiomycota*	*Agaricomycetes*	*Hymenochaetales*	*Rickenellaceae*	*Rickenella*	*mellea*
M2PP75.1	*Gelatoporia subvermispora* B	22434909	*Basidiomycota*	*Agaricomycetes*	*Polyporales*	*Gelatoporiaceae*	*Gelatoporia*	*subvermispora*
M98523.1	*Ustilago maydis*	8430103	*Basidiomycota*	*Ustilaginomycetes*	*Ustilaginales*	*Ustilaginaceae*	*Ustilago*	*maydis*
TPX56980.1	*Powellomyces hirtus*	31209237	*Chytridiomycota*	*Chytridiomycetes*	*Spizellomycetales*	*Powellomycetaceae*	*Powellomyces*	*hirtus*

Table 2.2. List of fungal species used in this study to build the Hidden Markov model for the monooxygenase-encoding sequences. The taxa in blue belong to phylum *Ascomycota*, while the green and yellow row(s) contain species from *Basidiomycota* and *Chytridiomycota* phyla.

2.3.3 Other siderophores

Beyond the biosynthetic gene clusters specifically associated with siderophores that we identified in our incubation using mass spectrometry, we discovered additional putative NRPSs and NISs for siderophore production (Table 2.3 and Figure 2.10). The following results should be interpreted with caution. Many of our contigs containing these other biosynthetic gene clusters were fragmented, making product predictions difficult. Furthermore, the presence of genes does not constitute expression. For these reasons, we advise our readers to be judicious when drawing conclusions from our findings and to consider the need for further experiments to validate functionality.

Despite the above limitations, our analysis yielded several noteworthy complete and incomplete siderophore-producing BGCs. We found the genes that are responsible for synthesizing several iron chelating moieties—1,1-dimethyl-3-amino-1,2,3,4-tetrahydro-7,8-dihydroxy-quinoline (dmaq), β-hydroxyaspartate, 2,3-dihydroxybenzoate (2,3-DHB), salicylate, hydroxamate, and 3,4- dihydroxybenzoate (3,4-DHB) (Table 2.3 and Figure 2.10). We analyzed the clusters with these genes and identified a few known siderophore candidates. For instance, we found nine MAGs containing NIS BGCs for synthesizing petrobactin-like and roseobactin-like siderophores, both of which belong to the same family of siderophores. Our taxonomic assignments of these MAGs were consistent with a recent study which determined that the petrobactin biosynthetic pathway is widespread across the global ocean, including the North Atlantic Ocean (Manck et al., 2022).

BGC type	Mag ID	Phylum	Class	Order	Family	Genus	Contig ID	Approx. BGC length	Chelating moiety	Related siderophore
NRPS	EAEBEC.89	p__Cyanobacteriota	c__Cyanobacteriia	o__Elainellales	f__Elainellaceae	g__JAAHGY01	k141_7083247	n.d.	Dmaq	Anachelin
	EB.53	p__Cyanobacteriota	c__Cyanobacteriia	o__Phormidesmiales	f__Phormidesmiaceae	g__Leptothoe	k141_2333413	n.d.	β-hydroxyaspartate	novel
	EB.39	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Stappiaceae	g__Roseibium	k141_2578959	n.d.	2,3DHB	Agrobactin
	EC.93	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Stappiaceae	g__Roseibium	k141_2734867	17723	2,3DHB	Vibriobactin
	EC.2	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Cognatishimia	k141_479736	7266	2,3DHB	Azotochelin
	EC.86	p__Pseudomonadota	c__Alphaproteobacteria	o__UBA8366	f__GCA-2696645	g__Curvivirga	k141_634868	19483	2,3DHB	Mediterraneabactin
	EAEBEC.155	p__Pseudomonadota	c__Gammaproteobacteria	o__Arenicellales	f__Arenicellaceae	g__Arenicella	k141_4762865	19071	β-hydroxyaspartate	novel
	EC.54	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	f__Alteromonadaceae	k141_3103393	n.d.	Salicylate and hydroxamate	novel
	EC.88	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	g__M269	k141_3118934	n.d.	β-hydroxyaspartate	n.d.
	EC.8	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Marinomonadaceae	g__Marinomonas	k141_5069228	17518	2,3DHB	Frederiksenibactin
NIS	EAEBEC.97	p__Bacteroidota	c__Bacteroidia	o__Cytophagales	f__Thermoflexibacteraceae	g__UBA2561	k141_7791133	8911	Hydroxamate	Bisucaberin
	EAEBEC.50	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Aquimarina	k141_3812872	8941	Hydroxamate	Bisucaberin
	EC.158	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Aquimarina	k141_2908304	9027	Hydroxamate	Bisucaberin
	EC.41	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Tenacibaculum	k141_468369	8836	Hydroxamate	Bisucaberin
	EC.42	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Tenacibaculum	k141_245462	8862	Hydroxamate	Bisucaberin
	CB.3	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Stappiaceae	g__Roseibium	k141_3575314	5646	3,4DHB	Petrobactin
	EB.125	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__188UL27-1	k141_3915043	n.d.	3,4DHB	Petrobactin
	EA.11	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Cognatishimia	k141_724014	6039	3,4DHB	Roseobactin
	EB.106	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Epibacterium	k141_1905805	7290	3,4DHB	Roseobactin
	EB.109	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Epibacterium	k141_3843757	4537	3,4DHB	Roseobactin
	CB.7	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Tateyamaria	k141_4177702	6855	3,4DHB	Petrobactin
	EAEBEC.167	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	f__Alteromonadaceae	k141_6940242	12236	Hydroxamate	Baumannoferrin
	EC.69	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	f__Alteromonadaceae	k141_4540496	6267	3,4DHB	Petrobactin
	EC.53	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Kangiellaceae	g__Aliikangiella	k141_1362610	6774	3,4DHB	Petrobactin
	EC.134	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__DT-91	g__DT-91	k141_5078845	6260	3,4DHB	Petrobactin

Table 2.3. Putative chelating moieties determined by the presence of their respective biosynthetic genes in our metagenome-assembled genomes (MAGs). Taxonomic classifications, contig identifications, and approximate gene cluster sizes [bp] are also included for each MAG. 2,3-DHB and 3,4-DHB stand for 2,3-dihydroxybenzoate and 3,4-dihydroxybenzoate, respectively. Dmaq is the short form of 1,1-dimethyl-3-amino-1,2,3,4-tetrahydro-7,8-dihydroxy-quinoline

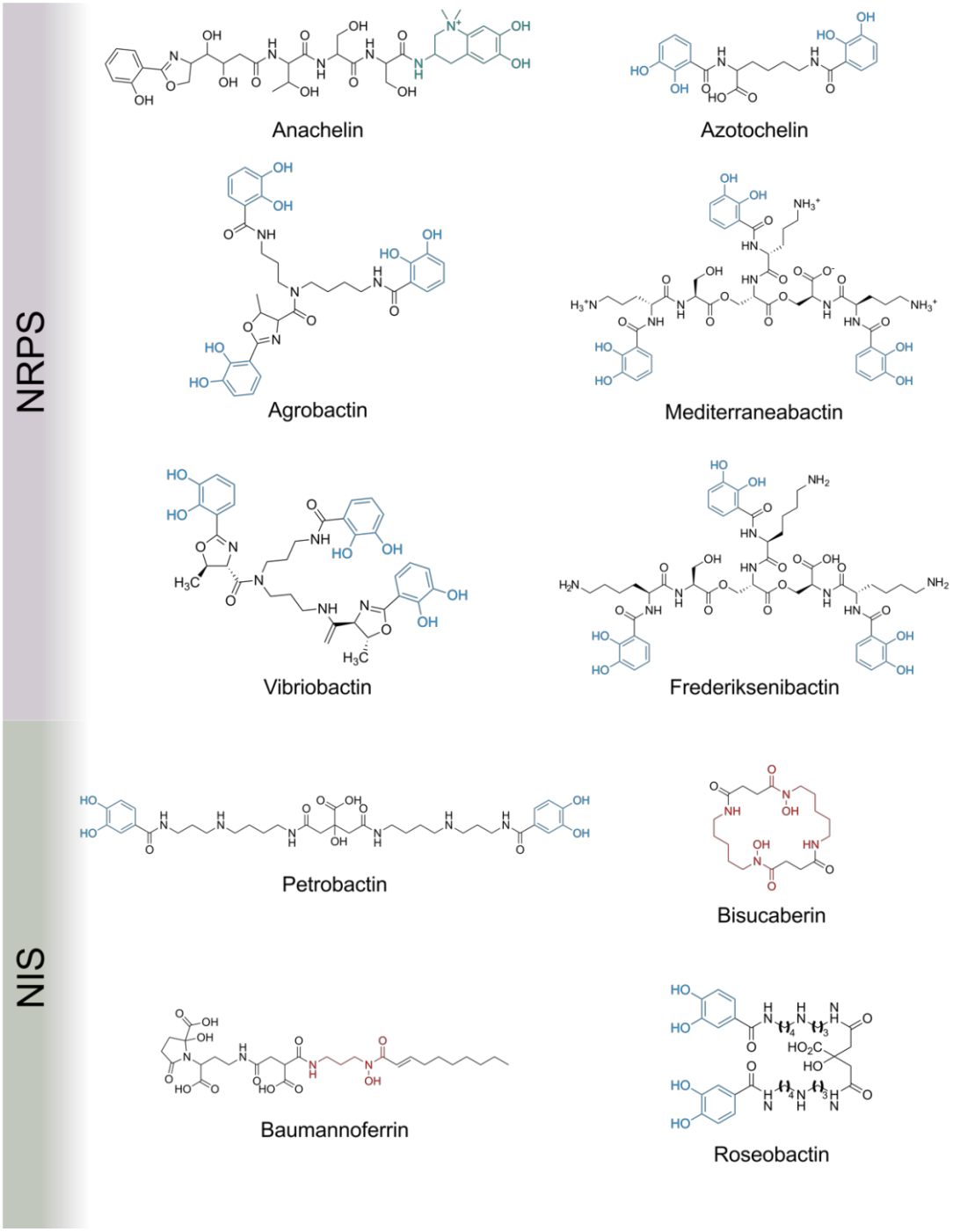


Figure 2.10. The chemical structures of the siderophores listed in the 'Related siderophores' column of Table 2.3. Please note that these structures are for example siderophores only. We are not proposing that the representative bacteria produce these siderophores but rather postulating that they may produce related siderophores with the chelating moieties of these predicted siderophores.

2.4 Conclusions

In this study, we investigated the metabolic capabilities of microbes associated with pelagic *Sargassum*, focusing on the strategies they employ to acquire iron from the seawater through siderophore production. Using incubation experiments, we show that some of these microorganisms are able to produce siderophores such as ferrioxamines, amphibactins, and ferrichromes. The former two siderophores are known to be bacterial in origin, while the latter is made by fungi. We complemented our findings using metagenomic data, and we were able to assemble high and medium quality metagenome-assembled genomes. Using these data, we were able find a single representative genome of the *Cellvibrionaceae* family that contained a complete biosynthetic gene cluster for making ferrioxamines. Furthermore, we found evidence indicating that the closest relative of an organism, capable of producing amphibactin, belong to the genus *Vibrio*. We did not find any fungal genes involved in the synthesis of ferrichromes.

The production of siderophores by microbes associated with the surface of Sargassum may facilitate the uptake of insoluble iron (Fe) by *Sargassum*. For instance, a reductive mechanism might occur at the interface between *Sargassum* and its microbial symbionts' cell surfaces. In this mechanism, iron could be reduced by membrane-bound reductases, allowing Fe^{2+} to be transported into the cell. Furthermore, it is plausible that siderophore-complexed iron is more photolabile, thereby becoming more bioavailable to both microbes and their macroalgal hosts, as observed in certain *Marinobacter* species (Amin et al., 2012). Furthermore, siderophores have been implicated in enhanced iron uptake rates by microalgae when the alga is co-cultured with siderophore-producing bacteria (Amin et al., 2009). Our results demonstrate the significant role that host-associated microbes potentially play in the

assimilation of new iron via siderophore production, thereby making it bioavailable to marine ecosystems.

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Chapter 3. Phosphonate utilization by free-living bacteria inside and outside the *Sargassum* patch

Taruna A. Schuelke, Chance English, Craig Carlson, Elizabeth Wilbanks

3.1 Introduction

3.1.1 DOP is a significant source of phosphorus in oligotrophic marine environments

Phosphorus (P) is an instrumental element in various cellular processes such as energy storage, signal transduction pathways, and nucleic acid biosynthesis, among others (Gasol & Kirchman, 2018). Unlike nitrogen fixation, there is no equivalent process in the phosphorus cycle; hence, atmospheric deposition and continental weathering serve as the primary sources of new phosphorus entering the ocean (Dyhrman, 2018). In contrast, burial in marine sediments is the main sink for phosphorus after it has been converted from a dissolved form to a particulate form. This process significantly influences the oceanic inventory of phosphorus, which is mostly comprised of dissolved forms of this vital element (Ruttenberg & Berner, 1993). Of these forms, dissolved organic phosphorus (DOP) often surpasses the inorganic pool of phosphorus in most open ocean settings (Karl & Björkman, 2015). An example of such environment is the oligotrophic North Atlantic Ocean where the inorganic phosphorus concentrations are frequently less than 10 nM and can dip below 1 nM (Cavender-Bares et al., 2001), and more than 80% of the total dissolved forms of phosphorus can be composed of DOP (Lomas et al., 2010). In such habitats, DOP serves as a significant source of phosphorus for phytoplankton and bacteria (Lomas et al., 2010).

It is well-established that the marine DOP pool consists of various organic compounds; however, identifying all these constituents is an active area of research. In fact, it has been postulated that less than half of the total DOP can be classified into constituents

using the current methods (Karl & Björkman, 2015). Despite the limitation in classification methods, we know that phosphoesters and phosphonates account for ~75% and ~25%, respectively, of the ultrafiltered DOP across different oceanic regions (*e.g.*, the Pacific Ocean, the North Sea, and the North Atlantic Ocean) (Kolowith et al., 2001; Young & Ingall, 2010). Phosphoesters contain ester bonds (C-O-P) that are commonly found in cellular biochemicals such as RNA, DNA, ATP, and lipids (Dyhrman, 2018). On the other hand, phosphonates are biogenic compounds defined by the presence of the highly stable carbon-phosphorus (C-P) bond which makes them resistant to chemical hydrolysis (Horsman & Zechel, 2017; Kafarski, 2020; Kolowith et al., 2001; Villarreal-Chiu et al., 2012). Phosphonates have important structural functions for marine bacterioplankton. For instance, they can conjugate to major components of cell membranes such as lipids, polysaccharides, and proteins, thereby conferring a robust cellular structure (Baumann et al., 1992; Quin & Quin, 2001). Furthermore, phosphonates can exhibit bioactive properties, such as the antibiotic Fosfomycin (Horsman & Zechel, 2017).

Here, we investigate the transport and catabolism of methylphosphonate (MPn) and 2-aminoethylphosphonate (2-AEP), two kinds of phosphonates known to have significant roles in marine environments. The presence of methane in oxygenated waters of the ocean has been explained by the degradation of MPn (Repeta et al., 2016), a phenomenon is known as the marine methane paradox because methane is typically generated by archaea during methanogenesis under anaerobic conditions. However, the breakdown of MPn by an enzyme called C-P lyase provides an alternative route for methane production in the surface waters of the open ocean (Carini et al., 2014; Metcalf et al., 2012; Repeta et al., 2016; Teikari et al., 2018). Furthermore, MPn, along with 2-hydroxyethylphosphonate (2-HEP) and

hydroxymethylphosphonate (HMP), contributed to approximately 21% of total P in the polysaccharide fraction of semi-labile dissolved organic matter (Repeta et al., 2016). In a similar manner, 2-AEP is also a critical phosphonate for bacterial productivity in oligotrophic oceans. A recent study discussed the ubiquitous nature of three bacterial transporters with high affinity to 2-AEP and emphasized the importance of this phosphonate in the marine phosphorus cycle (Murphy et al., 2021). Additionally, 2-AEP can be utilized as a source of nitrogen along with P (Horsman & Zechel, 2017). Considering the vital roles MPn and 2-AEP play in oceanic ecosystems, the primary objective of our study is to identify which free-living bacterial taxa within floating pelagic *Sargassum* patches are capable of degradation phosphonates and whether breakdown pathways are more prevalent inside versus outside these patches.

Pelagic *Sargassum* is a genus of macroalgae and is endemic to the oligotrophic waters of the North Atlantic Ocean. Previous research shows that *Sargassum* productivity can be limited by phosphorus availability (Cavender-Bares et al., 2001; Lomas et al., 2013; Wu et al., 2000). Yet, this macroalga thrives in this nutrient-limited environment. Here, we investigate whether *Sargassum* patches may be able to sustain their own growth by remineralizing phosphonate nutrients with the help of the microorganisms. In general, the ability to break down phosphonates is restricted to bacteria. However, new evidence shows that certain eukaryotic phytoplankton may also be able to degrade these organic compounds to use as a source of P (Whitney & Lomas, 2019). Although an exciting discovery, our understanding of the mechanisms behind phosphonate degradation in eukaryotes is limited and such pathways are primarily found in microorganisms. Thus, we are specifically interested in understanding how the free-living bacteria within pelagic *Sargassum* patches are

able to breakdown phosphonates in DOP and thereby, provide a source of inorganic phosphorus, a form readily available to *Sargassum*. To accomplish our goal, we sequenced the 16S rRNA marker gene and the metagenomes of free-living bacteria collected from inside and outside the pelagic *Sargassum* patches found in the P-limited Sargasso Sea and screened these data for organisms and genes known to be in the catabolism of phosphonates (Lomas et al., 2010). To our knowledge, no prior work has addressed the MPn and 2-AEP degradation potential of the microbes living in *Sargassum* communities. However, a recent investigation did demonstrate that *Sargassum's* epibionts can degrade methylphosphonate (Cox et al., 2023). While these findings highlight the potential role of *Sargassum*-associated microbes in phosphonate remineralization, the genetic mechanisms underlying this activity were not investigated.

3.1.2 The known phosphonates transport and degradation pathways

In P-limited conditions, proteins involved in the transport and degradation of phosphonates are generally under the control of the Pho regulon (Santos-Beneit, 2015). Once the regulon is activated, the phosphonate is transported across the membrane via one of two known systems—PhnSTUV and phnCDE (Santos-Beneit, 2015; Wanner, 1996). The enzyme products of PhnSTUV were initially elucidated in *Salmonella typhimurium* LT2 (Jiang et al., 1995; Kim et al., 2002). *PhnS* is the periplasmic substrate-binding domain, *phnT* is an ATP-binding cassette (ABC) transport domain, and *phnU* and *phnV* are the transmembrane proteins (Kim et al., 2002). The second transport system, PhnCDE, is part of the *phn* operon that encodes the multi-complex enzyme C-P lyase (discussed in detail below). This operon that is made up of 14 individual genes—*phnCDEFGHIJKLMNOP* (Metcalf & Wanner, 1993; Zeleznick et al., 1963). Similar to *phnSTUV*, the gene products of *phnC*, *phnD*, and

phnE encode an ABC transport domain, the periplasmic substrate-binding domain, and the transmembrane protein, respectively (Horsman & Zechel, 2017; Metcalf & Wanner, 1993). *PhnF* is a regulatory protein likely involved in transmembrane transport of the phosphonate substrates.

There are three pathways known to be utilized by microorganisms for catabolizing phosphonates (Figure 3.1) (Horsman & Zechel, 2017). These pathways are categorized as either hydrolytic, oxidative, or radical based on their specific mechanisms (Horsman & Zechel, 2017). The two known hydrolytic pathways are PhnWX and PhnWAY. *PhnW* and *phnX* genes encode 2-AEP-pyruvate transaminase (Kim et al., 2002) and phosphonoacetaldehyde hydrolase (Jiang et al., 1995), respectively. The first step in the hydrolytic pathways is the deamination of 2-AEP by transaminase to produce phosphonoacetaldehyde. After this reaction, the two pathways diverge. The phosphonoacetaldehyde product can be either converted to acetaldehyde and inorganic phosphate by phosphonoacetaldehyde hydrolase encoded by *phnX* or phosphonoacetaldehyde can be further transformed into phosphonoacetate by an NAD+-dependent phosphonoacetaldehyde dehydrogenase (phnY). Next, phosphonoacetate is hydrolyzed by phosphonoacetate hydrolase, encoded by *phnA*, to form acetate and inorganic phosphate (Agarwal et al., 2014; Borisova et al., 2011; Cooley et al., 2011).

PhnY'Z is the only known oxidative pathway comprising of α-ketoglutarate dioxygenase (phnY') and phosphohydrolase (phnZ) (McSorley et al., 2012; Van Staalduinen et al., 2014). The dioxygenase hydroxylates the α carbon of 2-AEP forming 2-amino-1-hydroxyethylphosphonate which is then oxidatively transformed to glycine and inorganic phosphate by the phosphohydrolase (McSorley et al., 2012). Beyond its involvement in the

breakdown of 2-AEP, *PhnY'Z* is found adjacent to genes encoding enzymes that decompose other P-containing molecules such as phosphite in *Prochlorococcus* species (Martinez et al. 2011). However, the precise function of phnY'Z in *Prochlorococcus* is unknown.

While hydrolytic and oxidative pathways described above are substrate specific (*i.e.*, 2-AEP) specific pathways, the radical mechanism found in the enzyme C-P lyase is promiscuous and can degrade several types of phosphonates (Horsman & Zechel, 2017). For instance, C-P lyase can cleave the C-P bond in 2-AEP as well as methylphosphonate (MPn). As mentioned previously, the C-P lyase is a multi-enzyme complex encoded by the *phn* operon that is made up of 14 individual genes—*phnCDEFGHIJKLMNOP* (Metcalf & Wanner, 1993; Zeleznick et al., 1963). This enzyme captures the phosphate by forming 5-phospho-α-D-ribosyl-1-diphosphate instead of releasing it as free inorganic phosphate (Horsman & Zechel, 2017). The gene products of *phnC*, *phnD*, *phnE*, and *phnF* take part in the transfer of phosphonates across the cell membrane. *PhnG* through *phnM* form enzymes with the actual lyase activity, and *phnN, phnO*, and *phnP* – are accessory proteins (Horsman & Zechel, 2017). We investigated all the *phn* genes described in this introductory section.

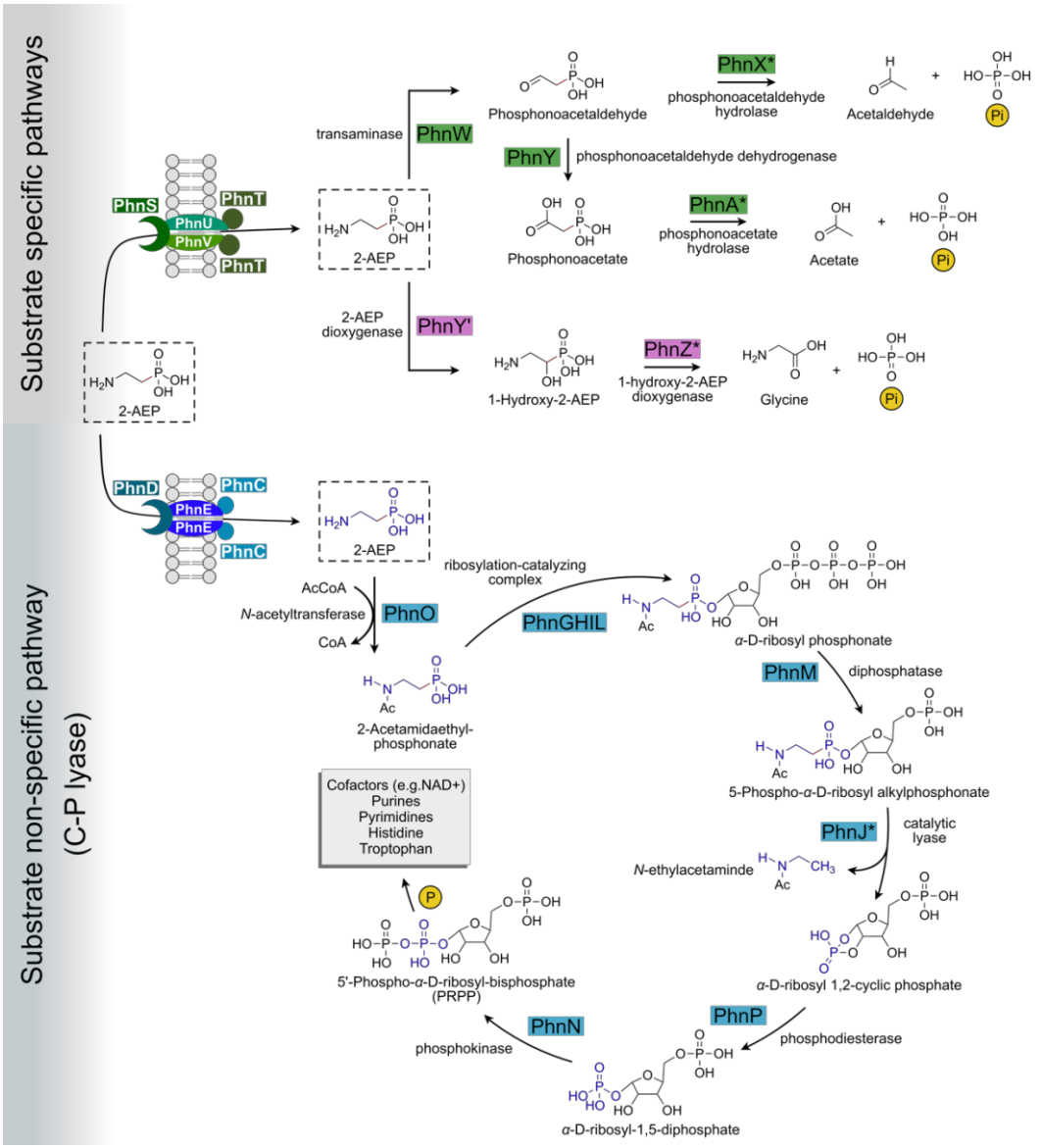


Figure 3.1 An overview of the transport and degradation pathways for 2-aminoethylphosphonate (2-AEP). 2-AEP can be taken up by a microbe via the PhnSTUV or the PhnCDE transport systems. Degradation of 2-AEP can occur via oxidative (PhnY'Z), hydrolytic (PhnWX and PhnWAY), and radical formation (CP-lyase) mechanisms. The *phnW* and *phnX* genes and the activity of their protein products were originally discovered in *Enterobacter aerogenes* IFO 12010 and *Salmonella typhimurium* LT2 (Jiang et al., 1995; K. S. Lee et al., 1992). The *phnA* gene sequence and expression analyses were first conducted in *Pseudomonas Fluorescens* 23F (Kulakova et al., 1997, 2001; McGrath et al., 1995). Lastly, the CP-lyase operon and the functional analysis of its gene products were studied in the model organism, *Escherichia coli* (Metcalf & Wanner, 1993).

3.2 Methods

3.2.1 Sampling free-living bacteria inside and outside the Sargassum patch

The sampling efforts were led by Chance English and Dr. Craig Carlson (affiliation Univ. of Calif., Santa Barbara) during fieldwork conducted in August 2021. We acid-washed and MilliQ-water rinsed 2L Teflon bottles that were used to collect surface seawater samples from inside (n=3) and outside (n=3) a *Sargassum* patch near Bermuda (32.29°N and 64.63°W) (Figure 3.2). Water samples were filtered onto 0.2 µm Teflon membranes immediately after collection and stored at -80ºC until DNA extraction could be performed. Additionally, English measured bacterial abundance, bacterial production, and dissolved organic carbon (DOC) concentrations in all six samples.

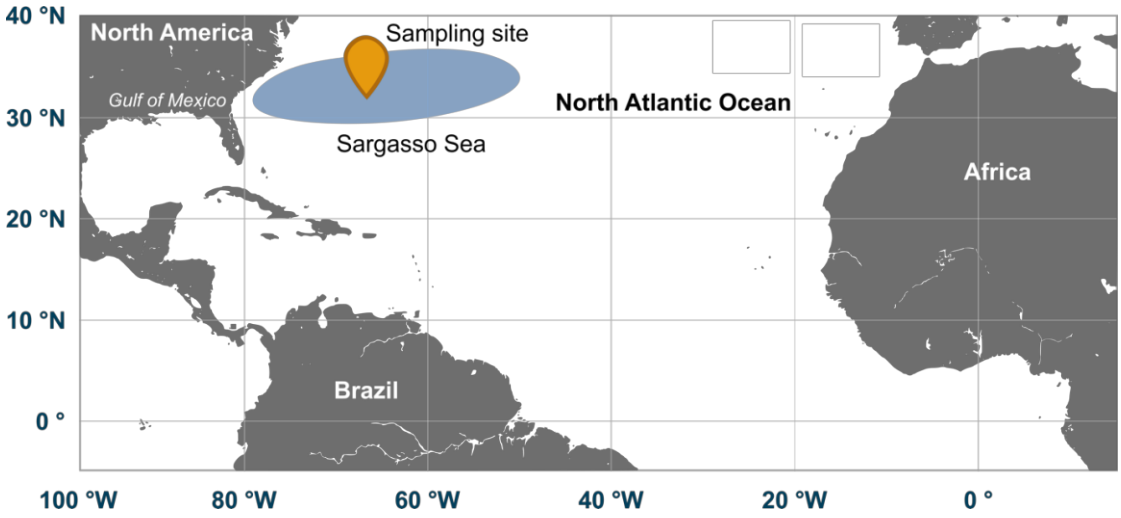


Figure 3.2. Map depicting the sampling location where Chance English collected three surface seawater samples from inside a pelagic *Sargassum* patch and three samples from outside the same patch.

3.2.2 Seawater analysis

Macronutrients in the surface seawater samples were analyzed at the Analytical Laboratory housed in the Marine Science Institute (Univ. of Calif., Santa Barbara). Specifically, concentrations of nitrite, nitrate plus nitrite, and ortho-phosphate were measured using a

continuous-flow technique (Lachat Instruments, QuikChem 8500 Series 2, Zellweger Analytics, Inc.). Bacterial abundance, production, and DOC were measured in the Carlson Laboratory at the Univ. of Calif., Santa Barbara.

3.2.3 DNA extraction and 16S rRNA library preparation & sequencing

DNA was extracted from all six filter samples by English following previously published methods (James et al., 2020). Briefly, bacterial cells filtered onto the 0.2 μm Teflon membranes were lysed with sucrose lysis buffer, 1% wt/vol sodium dodecyl sulfate and 0.2 mg mL^{-1} proteinase-K at 55°C for 2 hrs. DNA was extracted and purified using a spin-column method. 16S SSU rRNA (V4 region) amplicon libraries were generated following the Earth Microbiome Project protocol (Thompson et al., 2017). The forward and reverse 16S SSU rRNA primers were 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) (Apprill et al., 2015; Parada et al., 2016). Duplicate 25 μL PCR reactions per sample contained 12.5 μL Kapa Robust Hotstart ReadyMix, 1 μL each of the forward and reverse primers (10 μM), 1 μL (10 ng μL^{-1}) bovine albumin serum, 6.5 μL PCR water, and 3 μL genomic DNA. The DNA was initially denatured for 3 min at 95°C followed by 30 cycles of 30 sec at 95°C, 30 sec at 57°C, and 1 min at 72°C for primer annealing and amplifying DNA. A final extension step for 10 min at 72°C was also included. Negative control of PCR-grade water was included during PCR setup. The resulting libraries were purified, size-selected using magnetic beads, and sequenced on the MiSeq platform (Illumina) at the Biological Nanostructures Laboratory, Univ. of Calif., Santa Barbara. Duplicate reactions were combined together and cleaned and the resulting samples were normalized using SequalPrep plates (Invitrogen). Normalized amplicons were pooled at equal volumes and concentrated using Amicon Ultra 0.5 mL 30k centrifugal filters

(Millipore). English perform gel electrophoresis for each sample and the amplicons were gel extracted to eliminate nontarget bands (Qiagen Qiaquick). The final library was sequenced at University of California, Davis DNA Technologies Core on an Illumina MiSeq using PE250.

3.2.4 16S rRNA analysis

The DADA2 algorithm v.1.10.0 within the R package was used to resolve biological differences among sequences resulting in amplicon sequence variants (ASVs). Sequence quality control was performed through the identification and removal of chimeric sequences, sequences containing unknown (ambiguous) bases, and singleton reads. Next, the ASVs were taxonomically classified using the SILVA rRNA database v138 (515F-806R region only) which contained sequences clustered at 99% similarity. Sample by species matrix was constructed, and the taxonomic identifications were manually curated. Specifically, we removed all ASVs assigned to eukaryotes, chloroplasts, mitochondria, and unassigned taxa from our 16S samples by species matrix. The final 16S rRNA ASV sample by species matrix was analyzed in R using the Phyloseq package, and all statistical tests were also conducted in R. We also identified differentially abundant ASVs by comparing the mean relative abundances of ASVs inside and outside the *Sargassum* patch. ASVs which were at least twice as abundant inside the *Sargassum* patch than outside were considered differentially significant.

3.2.5 Metagenomic library preparation and sequencing

Metagenomic libraries using the six DNA samples were prepared by Schuelke. We first treated our DNA extracts with RNase I_f (NEB, cat. no. M0243S) following the manufacturer's recommendation. The RNase I_f was inactivated by incubating the samples at 70°C for 15 minutes. Next, the six RNA-free DNA extracts were sheared using the Covaris

M220. For each sample, 50 μL of the extract was used to shear the DNA in the microtube-50 AFA fiber screwcap (PN 520166). We followed the Covaris recommended protocol to accomplish the target fragment base pair peak of 200 bp. In general, the fragments tended to be approximately 100 bp larger than the target peak. The DNA fragment size distributions in each sample were assessed on Agilent TapeStation using the high sensitivity D5000 tape assay (cat. nos. 5067-5592 & 5067-5593). Libraries for sequencing were prepared using the ThruPLEX DNA-Seq HV kit by Takara Bio USA, Inc. (cat. no. R400741) following the company's protocol and were submitted to UC Davis Genome Center for sequencing on the Novaseq 6000 platform (Illumina).

3.2.6 Metagenomic assembly and annotation

Read quality for each sample was assessed using FastQC (Andrews et al., 2012). We used TrimGlore v0.6.10 for read quality control (https://github.com/FelixKrueger/TrimGalore). Adapters and reads were trimmed using the default minimum read length parameter and using a threshold phred score of 15 (Bolger et al., 2014). We constructed both single sample assemblies and co-assemblies with MEGAHIT v1.2.9 using all paired and unpaired reads that passed our filtering thresholds (Li et al., 2015). Next, we mapped our trimmed reads from each individual sample to each assembly and co-assembly to obtain differential sequencing coverage information using Bowtie 2 v2.4.4 and SAMtools v1.13.0 (Danecek et al., 2021; Langmead & Salzberg, 2012).

Contigs larger than 2,500 bp, from each assembly type, were binned into metagenome-assembled genomes (MAGs) using MetaBAT2 (D. D. Kang et al., 2019). We used CheckM v1.2.2 to determine assembly and competition statistics (Parks et al., 2015). We conducted taxonomic classification using GTDB-Tk v2.3.0 against the Genome

Taxonomy Database R214, and we dereplicated our MAGs with dRep v3.4.3 (Chaumeil et al., 2020; Olm et al., 2017). We used Prodigal v2.6.3 to predict genes within our MAGs and performed a targeted annotation of phosphonate degradation genes described in figure 3.1 and carbohydrate-active enzymes (CAZymes) with HMMER v3.3.2 using publicly available protein hidden Markov models of these genes (HMMs) (Cantarel et al., 2009; Finn et al., 2011; Hyatt et al., 2010).

3.3. Results and discussion

3.3.1 Bacterial abundance and production are higher inside the *Sargassum* patch than outside the patch

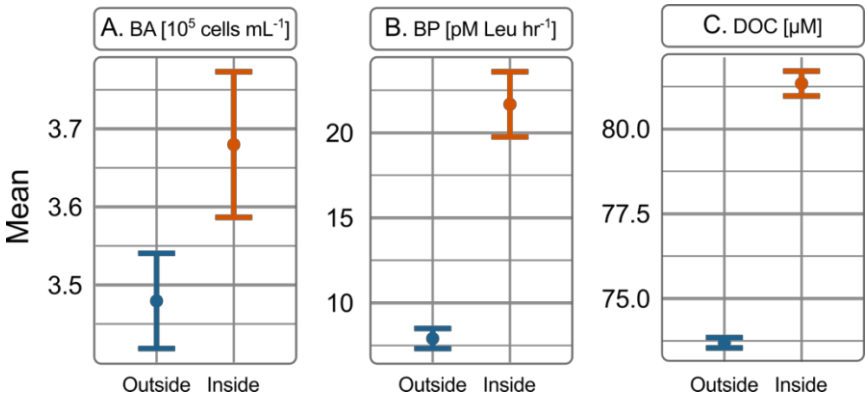


Figure 3.2. Mean values of (A) bacterial abundance (BA), (B) bacterial productivity (BP), and (C) dissolved organic carbon (DOC) from seawater samples collected outside and inside the *Sargassum* patch. Dots represent the mean values, and bars indicate the standard deviation based on three data points for each variable, except for bacterial abundance inside the *Sargassum* patch, which is based on two data points.

Macroalgae, including *Sargassum*, are a rich source of nutritious organic matter (Barrón et al., 2014; Filbee-Dexter & Scheibling, 2014; Powers et al., 2019b; Reed et al., 2015). Consequently, macroalgal communities are home to an ample and diverse set of microorganisms (Egan et al., 2013). Considering this, we measured the bacterial abundance, production, and DOC in seawater samples collected from outside and inside the *Sargassum*

patch. Our data show that bacteria were present in greater number and were more productive inside the patch where DOC level was also higher. However, these findings were not statistically significant, ($p>0.05$, Exact Permutation Test) which is likely a result of our small sample size. Nonetheless, the higher bacterial abundance and production within the *Sargassum* patch suggests enhanced microbial activity which is crucial in the generation and remineralization of organic matter. Bacteria likely facilitate the degradation of complex organic compounds, releasing essential nutrients that can be readily consumed by *Sargassum*.

3.3.2 Members of the *Pseudomonadales* order are more abundant inside the *Sargassum* patch than outside the patch

After detecting higher bacterial abundance and production inside the pelagic *Sargassum* patch, we sequenced the 16S rRNA gene of free-living microbial consortia from seawater samples that were collected from inside (n=3) and outside (n=3) the patch. After filtering out ASVs assigned to archaea, chloroplast, and mitochondria, a total of 310 16S rRNA ASVs remained. Our alpha diversity analysis suggests that *Sargassum* patches harbor a more diverse microbial community than seawater surrounding the patch (Figure 3.3A). The inside patch samples exhibit higher species richness, higher diversity, and lower evenness than the outside patch samples, suggesting that some microbes potentially dominate *Sargassum* patches. Furthermore, although the inside and outside samples are distributed separately in Figure 3.3B, we found that the β-diversity between the free-living bacterial communities inside a *Sargassum* patch do not differ from the free-living communities found in the surrounding seawater of the patch (Figure 3.3B; ANOSIM: p-value = 0.1, R = 0.2963). The

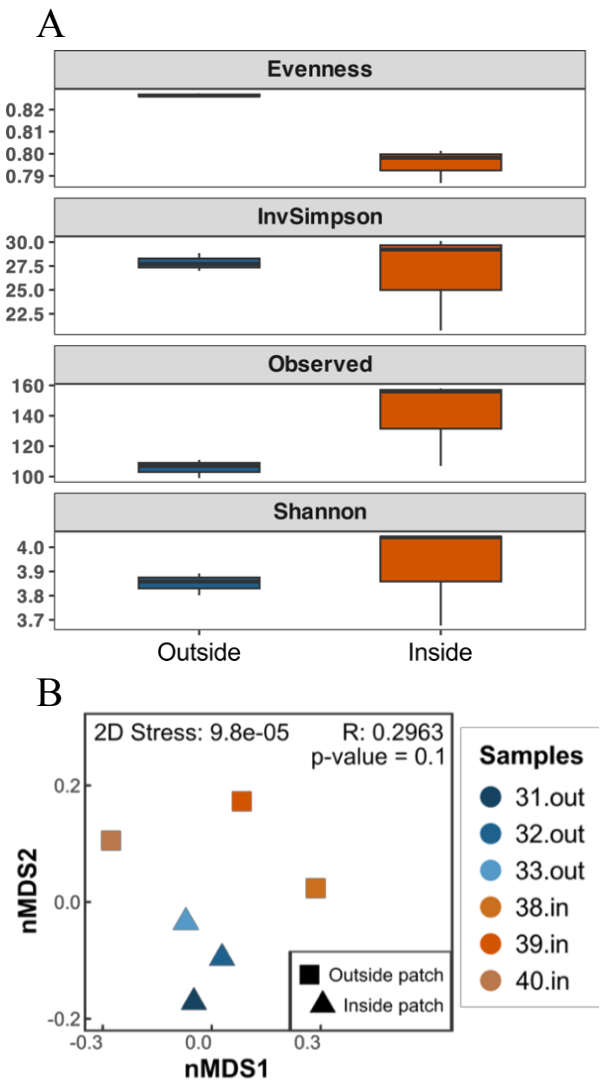


Figure 3.3. (A) Univariate descriptors of diversity associated with control and experimental samples. All p-values are ≥ 0.05. (B) The beta diversity of microbial communities using weighted UniFrac distances. Each data point represents a microbial community sample, with warm colored points indicating samples from inside the *Sargassum* patch and cold colored points indicating samples from outside the patch. The distance between points reflects the phylogenetic dissimilarity between communities. An ANOSIM test revealed the two communities were not significantly different.

top three phyla were present at similar relative percentages outside and inside the patch (*Bacteroidota* [8% outside versus 7% inside], *Cyanobacteria* [10% outside versus 6% inside], and *Proteobacteria* [75% outside versus 82% inside].

Despite the overall similarity between the two microbial communities, we identified eight ASVs that were at least twice as abundant inside the *Sargassum* patch than outside (Table 3.1, Kruskal-Wallis test: p-value < 0.05 and Figure 3.4). Five out of eight ASVs belong to order *Pseudomonadales* while the other two were classified as members of *Enterobacterales* and *Flavobacteriales*. Three ASVs of *Pseudomonadales* were distinguished into two known families (*i.e.*, *Marinomonadaceae* and *Saccharospirillaceae*) while the taxonomy of the remaining two ASVs (*i.e.*, ASV11 and ASV52) were not further resolved. A BLAST search of ASV11 and ASV52 revealed that

they are closely related to *Reinekea thalattae* SSH23 (accession NR_180338.1, 94% identity) and *Pseudomonas marincola* AB251f (accession NR_117186.1, 93% identity), respectively. Furthermore, we found another amplicon sequence, ASV83, belonging to genus *Reinekea*. Taking this into consideration, the ASV cataloged within the genus *Reinekea* account for nearly 3% of the community. These results suggest that specific bacterial taxa, particularly those within the *Pseudomonadales* order, may have specialized roles in responding to the unique ecological conditions inside *Sargassum* patches (*e.g.*, phosphonate remineralization).

ASV ID	Outside (RA)	Inside (RA)	Order	Family	Genus
ASV11	0	2.75	o_*Pseudomonadales*	-	-
ASV51	0	0.50	o_*Enterobacterales*	f_*Vibrionaceae*	g_*Vibrio*
ASV52	0	0.31	o_*Pseudomonadales*	-	-
ASV58	0	0.35	o_*Pseudomonadales*	f_*Marinomonadaceae*	g_*Marinomonas*
ASV78	0	0.23	o_*Flavobacteriales*	f_*Flavobacteriaceae*	g_*Tenacibaculum*
ASV80	0	0.20	o_*Pseudomonadales*	f_*Marinomonadaceae*	g_*Marinomonas*
ASV83	0	0.10	o_*Pseudomonadales*	f_*Saccharospirillaceae*	g_*Reinekea*

Table 3.1. List of ASVs that are differentially more abundant inside the *Sargassum* patch than outside and their relative abundances (RA).

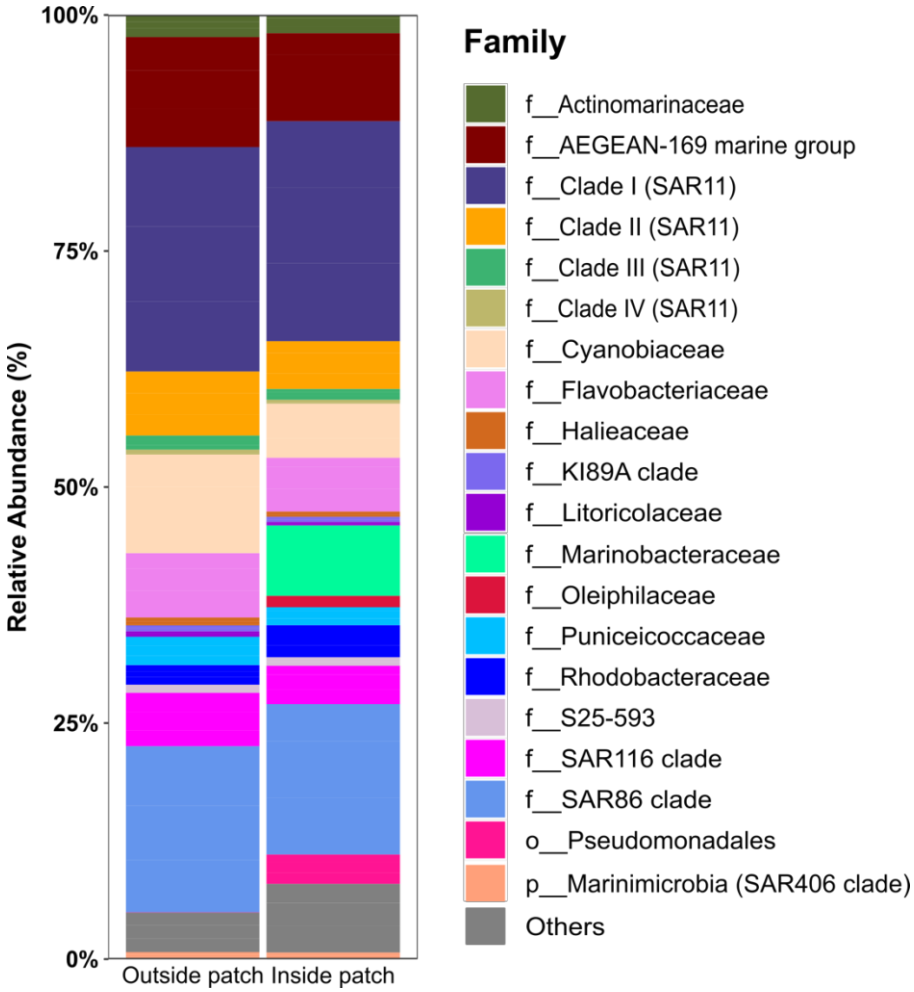


Figure 3.4. Relative abundance of free-living bacterial communities in seawater samples collected from inside and outside a pelagic *Sargassum* patch. Taxonomy of the top 20 most abundant families is shown. The low abundant taxa are grouped in the "others" category .

3.3.3 Brief overview of metagenomic assembled genomes

We generated metagenomic assembled genomes (MAGs) from the above six seawater samples collected from inside and outside the pelagic *Sargassum* patch. Approximately 1.4 billion high-quality read pairs were assembled and co-assembled to build a total of 2,181 MAGs. The genome dereplication process reduced this batch to a final set of 159 unique high and medium quality MAGs belonging to 35 different families represented by 11 bacterial phyla (Figure 3.5). The two most abundant phyla are *Pseudomonadota* and *Bacteroidota*. These two phyla are also represented in high proportions in our 16S rRNA gene data. The assembly statistics, completion matrices, and the taxonomic information for non-redundant, bacterial MAGs assembled from these samples are listed in table 3.2.

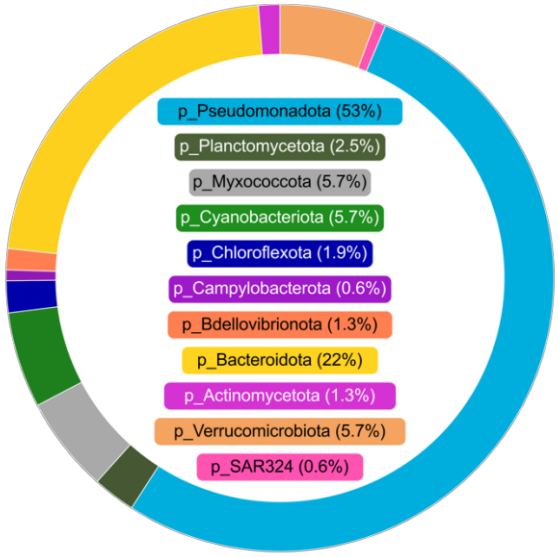


Figure 3.5. Percentages of phyla represented in the metagenomic assembled genomes.

MAG ID	Comp. (%)	Cont.	Qual.	Contig num.	Genome size (Mbp)	Longest contig (kbp)	N50 (kbp)	GC (%)	Phylum	Class	Order	Family	Genus	Species
MAG.1	70.41	1.28	Med	583	3.13	69.2	5.8	62.14	p__Actinomycetota	c__Acidimicrobiia	o__Acidimicrobiales	f__SHLQ01	g__SHLQ01	-
MAG.2	94.44	3.3	High	160	3.39	120.6	39.8	62.52	p__Actinomycetota	c__Acidimicrobiia	o__Acidimicrobiales	f__SHLQ01	g__SHLQ01	-
MAG.3	94.22	2.96	High	108	2.03	169.7	34.4	43.04	p__Bacteroidota	c__Bacteroidia	o__Chitinophagales	f__UBA8649	g__UBA8649	s__UBA8649 sp002862745
MAG.4	57.87	8.07	Med	149	0.75	14.1	5.4	44.78	p__Bacteroidota	c__Bacteroidia	o__Cytophagales	f__Amoebophilaceae	-	-
MAG.5	61.75	1.37	Med	85	0.72	33.3	10.7	50.66	p__Bacteroidota	c__Bacteroidia	o__Cytophagales	f__Amoebophilaceae	-	-
MAG.6	70.6	8.2	Med	119	0.99	35.9	10.5	48.76	p__Bacteroidota	c__Bacteroidia	o__Cytophagales	f__Amoebophilaceae	g__UBA8403	-
MAG.7	93.17	3.9	High	1034	8.34	50.8	9.8	40.11	p__Bacteroidota	c__Bacteroidia	o__Cytophagales	f__Microscillaceae	g__Microscilla	-
MAG.8	80.25	0.83	Med	531	2.86	21.1	5.8	31.86	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Aquimarina	-
MAG.9	93.01	1.47	High	70	1.37	131.7	50.4	30.46	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__GCA-2862715	s__GCA-2862715 sp002862715
MAG.10	88.6	2.57	Med	62	1.21	81.3	41.2	30.26	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Marisimplicoccus	s__Marisimplicoccus sp902519955
MAG.11	57.95	5.97	Med	159	1.04	25.6	8.3	30.33	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Marisimplicoccus	s__Marisimplicoccus sp902598525
MAG.12	73.87	2.09	Med	84	1.35	78.4	28.3	30.41	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__MED-G13	s__MED-G13 sp002691265
MAG.13	85.66	1.84	Med	132	1.24	45.8	14.6	31.27	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__MED-G14	s__MED-G14 sp002697465
MAG.14	89.66	6.97	Med	122	1.24	60.0	15.3	29.67	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__MED-G14	s__MED-G14 sp004321735
MAG.15	98.53	0	High	80	2.22	196.4	48.3	36.03	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__MS024-2A	s__MS024-2A sp002457295
MAG.16	91.54	1.57	High	75	2.00	165.3	50.9	38.17	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__MS024-2A	s__MS024-2A sp902546725
MAG.17	79.91	4.87	Med	487	2.87	25.6	7.0	33.11	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Ochrovirga	-
MAG.18	67.31	4.77	Med	422	2.16	16.4	5.6	34.42	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Pustulibacterium	-
MAG.19	91.5	3.84	High	426	4.03	56.2	13.0	30.34	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__Tenacibaculum	-
MAG.20	61.4	3.68	Med	98	1.04	43.2	18.5	30.93	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__TMED96	s__TMED96 sp002171475
MAG.21	85.46	1.21	Med	34	1.44	255.9	81.7	39.89	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__UBA724	s__UBA724 sp902536935
MAG.22	70.83	0.41	Med	16	1.33	344.3	323.8	39.51	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__UBA724	s__UBA724 sp902586905
MAG.23	92.29	2.85	High	41	1.57	218.3	83.7	39.93	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__UBA7446	-
MAG.24	81.17	10.08	Med	199	1.43	41.8	8.7	39.76	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__UBA7446	s__UBA7446 sp002470745
MAG.25	73.56	1.91	Med	125	1.10	43.0	10.4	42.59	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__UBA7446	s__UBA7446 sp002862705
MAG.26	99.05	0.16	High	23	2.54	368.8	169.8	39.65	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__UBA8316	-
MAG.27	89.33	0	Med	7	1.91	618.5	519.6	37.34	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Flavobacteriaceae	g__UBA8316	s__UBA8316 sp913030895
MAG.28	75.86	0	Med	74	1.94	234.2	42.3	29.85	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Parvicellaceae	g__MED-G20	s__MED-G20 sp002691605
MAG.29	98.39	1.18	High	327	3.37	98.9	15.2	37.13	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__Schleiferiaceae	g__Owenweeksia	-
MAG.30	97.85	0	High	28	2.29	419.6	157.6	55.97	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__UA16	g__UA16	s__UA16 sp002863125
MAG.31	94.35	6.17	Med	148	2.17	112.3	25.4	58.15	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__UA16	g__UBA11663	s__UBA11663 sp002863145
MAG.32	94.18	0	High	34	2.14	293.1	90.8	60.84	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__UA16	g__UBA8752	s__UBA8752 sp002172485
MAG.33	91.13	0.09	High	61	2.29	198.3	81.8	62.55	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__UA16	g__UBA8752	s__UBA8752 sp002862785
MAG.34	86.36	1.08	Med	86	1.51	95.2	33.1	26.63	p__Bacteroidota	c__Bacteroidia	o__Flavobacteriales	f__UBA7430	g__GCA-2692065	s__GCA-2692065 sp902551855
MAG.35	61.21	0	Med	243	1.56	44.8	8.0	40.96	p__Bacteroidota	c__Rhodothermia	o__Balneolales	f__Balneolaceae	g__UBA1275	s__UBA1275 sp002457365
MAG.36	51.18	8.93	Med	336	1.61	18.9	5.3	39.4	p__Bacteroidota	c__Rhodothermia	o__Balneolales	f__Balneolaceae	g__UBA1275	s__UBA1275 sp902529115
MAG.37	62.07	0	Med	221	1.63	28.6	9.4	39.94	p__Bacteroidota	c__Rhodothermia	o__Balneolales	f__Balneolaceae	g__UBA1275	s__UBA1275 sp902529365
MAG.38	59.01	1.83	Med	523	2.49	21.3	5.0	39.49	p__Bdellovibrionota	c__Bacteriovoracia	o__Bacteriovoracales	f__Bacteriovoracaceae	g__GCA-2712005	-
MAG.39	82.33	2.98	Med	355	2.60	35.0	8.7	43.8	p__Bdellovibrionota	c__Bdellovibrionia	o__Bdellovibrionales	f__UBA6776	-	-
MAG.40	90.53	2.24	High	373	2.47	31.9	8.2	29.93	p__Campylobacterota	c__Campylobacteria	o__Campylobacterales	f__Arcobacteraceae	-	-
MAG.41	73.18	1.27	Med	526	3.95	39.4	9.2	49.43	p__Chloroflexota	c__Anaerolineae	o__Promineofilales	f__Promineofilaceae	g__JAMLHW01	-
MAG.42	49.7	0.91	Med	409	2.52	31.5	6.6	48.06	p__Chloroflexota	c__Anaerolineae	o__Promineofilales	f__Promineofilaceae	g__JAMLHW01	-
MAG.43	93.43	5	High	476	5.93	87.5	19.4	47.8	p__Chloroflexota	c__Anaerolineae	o__Promineofilales	f__Promineofilaceae	g__WTJY01	-
MAG.44	90.1	0.76	High	576	6.10	44.1	14.3	33.92	p__Cyanobacteriota	c__Cyanobacteriia	o__Cyanobacteriales	f__Microcoleaceae	g__Trichodesmium	s__Trichodesmium erythraeum
MAG.45	94.65	4.44	High	793	7.39	42.5	12.4	35.02	p__Cyanobacteriota	c__Cyanobacteriia	o__Cyanobacteriales	f__Microcoleaceae	g__Trichodesmium	s__Trichodesmium sp023356605
MAG.46	71.8	2.33	Med	1050	5.23	22.8	5.3	37.6	p__Cyanobacteriota	c__Cyanobacteriia	o__Cyanobacteriales	f__Nostocaceae	g__Mastigocoleus	-
MAG.47	59.64	8.33	Med	857	3.49	21.5	4.1	39.99	p__Cyanobacteriota	c__Cyanobacteriia	o__Cyanobacteriales	f__Xenococcaceae	g__Hyella	-
MAG.48	77.24	0.82	Med	455	5.60	107.7	17.3	48.92	p__Cyanobacteriota	c__Cyanobacteriia	o__Elainellales	f__Elainellaceae	g__JAAHGY01	-
MAG.49	50.47	0.27	Med	668	3.21	16.2	5.1	47.99	p__Cyanobacteriota	c__Cyanobacteriia	o__Elainellales	f__Elainellaceae	g__JAAHGY01	-
MAG.50	87.06	4.89	Med	195	1.83	78.2	12.8	63.7	p__Cyanobacteriota	c__Cyanobacteriia	o__PCC-6307	f__Cyanobiaceae	g__Parasynechococcus	s__Parasynechococcus sp000737575
MAG.51	83.83	2.72	Med	157	2.02	58.7	19.0	58	p__Cyanobacteriota	c__Cyanobacteriia	o__PCC-6307	f__Cyanobiaceae	g__Parasynechococcus	s__Parasynechococcus sp002700765
MAG.52	81.73	2.72	Med	185	1.73	87.3	13.4	62.31	p__Cyanobacteriota	c__Cyanobacteriia	o__PCC-6307	f__Cyanobiaceae	g__Parasynechococcus	s__Parasynechococcus sp004212765
MAG.53	69.7	4.44	Med	407	3.70	51.6	11.6	54.4	p__Myxococcota	c__Bradymonadia	o__UBA7976	f__UBA1532	-	-
MAG.54	92.58	3.23	High	75	5.32	437.4	141.9	58.91	p__Myxococcota	c__Bradymonadia	o__UBA7976	f__UBA1532	g__UBA1532	-
MAG.55	76.4	3.36	Med	568	4.44	57.8	9.7	68.55	p__Myxococcota	c__UBA796	o__UBA796	f__UBA2385	-	-
MAG.56	87.41	3.36	Med	496	5.22	108.7	14.7	47.01	p__Myxococcota	c__UBA796	o__UBA796	f__UBA796	-	-
MAG.57	88.76	1.73	Med	324	5.13	210.8	24.6	50.88	p__Myxococcota	c__UBA796	o__UBA796	f__UBA796	g__CAJWHX01	-
MAG.58	92.69	2.1	High	173	6.00	183.5	51.7	50.58	p__Myxococcota	c__UBA796	o__UBA796	f__UBA796	g__CAJWHX01	-
MAG.59	83.52	1.31	Med	515	6.34	61.9	18.5	39.42	p__Myxococcota	c__UBA796	o__UBA796	f__UBA796	g__GCA-2683315	-
MAG.60	83.13	5.04	Med	447	4.15	67.3	11.6	56.9	p__Myxococcota	c__UBA796	o__UBA796	f__UBA796	g__UBA796	-

MAG ID	Comp. (%)	Cont.	Qual.	Contig num.	Genome size (Mbp)	Longest contig (kbp)	N50 (kbp)	GC (%)	Phylum	Class	Order	Family	Genus	Species
MAG.61	92.86	2.52	High	48	4.27	345.2	189.6	62.8	p__Myxococcota	c__UBA796	o__UBA796	f__UBA796	g__UBA796	-
MAG.62	68.28	1.2	Med	431	2.25	23.5	5.8	68.04	p__Planctomycetota	c__Phycisphaerae	o__Phycisphaerales	f__SM1A02	g__GCA-002862325	-
MAG.63	100	0.57	High	39	2.65	285.1	173.9	68.01	p__Planctomycetota	c__Phycisphaerae	o__Phycisphaerales	f__SM1A02	g__UBA8087	s__UBA8087 sp002862305
MAG.64	59.55	0	Med	158	1.17	35.9	9.2	56.45	p__Planctomycetota	c__Phycisphaerae	o__Phycisphaerales	f__SM1A02	g__UBA8653	-
MAG.65	76.72	0.57	Med	272	1.80	29.6	7.6	57.15	p__Planctomycetota	c__Phycisphaerae	o__Phycisphaerales	f__SM1A02	g__UBA8653	s__UBA8653 sp002862275
MAG.66	49.61	0	Med	367	1.70	16.9	4.9	48.01	p__Pseudomonadota	c__Alphaproteobacteria	o__Caulobacterales	f__Maricaulaceae	g__JACOMS01	-
MAG.67	51.37	9.52	Med	399	1.62	11.2	4.1	47.52	p__Pseudomonadota	c__Alphaproteobacteria	o__Caulobacterales	f__Maricaulaceae	g__Litorimonas	-
MAG.68	67.1	4.23	Med	897	4.76	23.3	5.7	58.73	p__Pseudomonadota	c__Alphaproteobacteria	o__Geminicoccales	f__Geminicoccaceae	-	-
MAG.69	65.93	5.36	Med	252	1.32	24.2	5.8	41.7	p__Pseudomonadota	c__Alphaproteobacteria	o__Micavibrionales	f__Micavibrionaceae	g__UBA1664	-
MAG.70	93.33	0.29	High	39	1.71	236.6	84.0	45.99	p__Pseudomonadota	c__Alphaproteobacteria	o__Parvibaculales	f__RS24	g__Micropelagos	s__Micropelagos sp002450335
MAG.71	88.33	1.32	Med	130	1.99	84.3	25.4	44.45	p__Pseudomonadota	c__Alphaproteobacteria	o__Parvibaculales	f__RS24	g__Micropelagos	s__Micropelagos thuwalensis
MAG.72	51.38	2.1	Med	246	1.18	21.9	4.9	55.1	p__Pseudomonadota	c__Alphaproteobacteria	o__Parvibaculales	f__RS24	g__UBA7378	-
MAG.73	98.12	0.22	High	35	1.82	288.1	80.0	56.22	p__Pseudomonadota	c__Alphaproteobacteria	o__Parvibaculales	f__RS24	g__UBA7378	s__UBA7378 sp913051715
MAG.74	59.04	0	Med	17	0.66	116.6	83.8	29.4	p__Pseudomonadota	c__Alphaproteobacteria	o__Pelagibacterales	f__Pelagibacteraceae	g__HIMB114	s__HIMB114 sp002171085
MAG.75	57.87	1.31	Med	195	1.30	34.1	7.8	46.52	p__Pseudomonadota	c__Alphaproteobacteria	o__Puniceispirillales	f__Puniceispirillaceae	g__HIMB100	s__HIMB100 sp002700485
MAG.76	98.85	0	High	43	2.14	178.4	74.3	40.63	p__Pseudomonadota	c__Alphaproteobacteria	o__Puniceispirillales	f__Puniceispirillaceae	g__UBA5951	s__UBA5951 sp016779005
MAG.77	55.69	9.15	Med	299	1.38	20.3	5.0	61.77	p__Pseudomonadota	c__Alphaproteobacteria	o__Puniceispirillales	f__Puniceispirillaceae	g__UBA8309	-
MAG.78	64.97	1.72	Med	139	1.83	89.8	23.5	61.81	p__Pseudomonadota	c__Alphaproteobacteria	o__Puniceispirillales	f__Puniceispirillaceae	g__UBA8309	-
MAG.79	97.58	7.7	Med	121	2.53	182.0	34.9	60.41	p__Pseudomonadota	c__Alphaproteobacteria	o__Puniceispirillales	f__Puniceispirillaceae	g__UBA8309	s__UBA8309 sp002457745
MAG.80	96.24	0	High	140	2.44	95.3	26.6	56.91	p__Pseudomonadota	c__Alphaproteobacteria	o__Puniceispirillales	f__Puniceispirillaceae	g__UBA8309	s__UBA8309 sp016780765
MAG.81	88.3	2.77	Med	171	2.38	88.7	23.7	62.5	p__Pseudomonadota	c__Alphaproteobacteria	o__Puniceispirillales	f__Puniceispirillaceae	g__UBA8309	s__UBA8309 sp913048595
MAG.82	96.39	0.47	High	110	2.02	144.3	29.5	56.98	p__Pseudomonadota	c__Alphaproteobacteria	o__Puniceispirillales	f__UBA1172	g__UBA1172	s__UBA1172 sp002457135
MAG.83	97.1	1.32	High	239	5.08	148.0	36.2	53.54	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhizobiales	f__Hyphomicrobiaceae	g__GCA-2689605	s__GCA-2689605 sp002689605
MAG.84	89.02	2.33	Med	139	1.93	82.2	24.0	37.29	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__CACIJG01	s__CACIJG01 sp003331935
MAG.85	90.19	2.21	High	150	3.28	115.6	38.8	46.17	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__LFER01	-
MAG.86	64.3	2.32	Med	376	1.91	21.8	5.4	51.22	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__LGRT01	s__LGRT01 sp016778765
MAG.87	66.45	3.09	Med	479	2.59	22.1	6.1	59.1	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Planktotalea	-
MAG.88	61.99	4.47	Med	713	3.30	17.7	4.8	63.26	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__Thermohalobaculum	-
MAG.89	92.34	2.29	High	315	3.11	52.9	13.8	55.08	p__Pseudomonadota	c__Alphaproteobacteria	o__Rhodobacterales	f__Rhodobacteraceae	g__WX04	-
MAG.91	100	0	High	26	1.62	279.5	114.5	46.75	p__Pseudomonadota	c__Alphaproteobacteria	o__Rickettsiales	-	-	-
MAG.90	93.37	0.55	High	176	1.55	43.3	10.0	47.89	p__Pseudomonadota	c__Alphaproteobacteria	o__Rickettsiales	f__RI-342	-	-
MAG.92	97.56	0.22	High	110	2.95	168.7	49.0	63.69	p__Pseudomonadota	c__Alphaproteobacteria	o__Thalassobaculales	f__Thalassobaculaceae	g__TMED26	s__TMED26 sp002694075
MAG.93	99.57	0.45	High	137	3.01	106.9	34.4	55.22	p__Pseudomonadota	c__Alphaproteobacteria	o__Thalassobaculales	f__Thalassobaculaceae	g__TMED26	s__TMED26 sp003280605
MAG.94	86.81	6.04	Med	55	1.38	184.2	79.7	29.97	p__Pseudomonadota	c__Alphaproteobacteria	o__TMED127	f__TMED127	g__MED-G10	s__MED-G10 sp002457065
MAG.95	91.6	0	High	69	1.40	182.4	38.5	35.76	p__Pseudomonadota	c__Alphaproteobacteria	o__TMED25	f__TMED25	g__TMED25	s__TMED25 sp902633855
MAG.96	74.78	3.48	Med	68	2.37	255.2	61.3	59.07	p__Pseudomonadota	c__Alphaproteobacteria	o__UBA7985	f__UBA7985	g__UBA7985	s__UBA7985 sp002690455
MAG.97	96.3	0.43	High	71	2.62	233.2	86.9	64.49	p__Pseudomonadota	c__Alphaproteobacteria	o__UBA7985	f__UBA7985	g__UBA7985	s__UBA7985 sp002708995
MAG.98	85.32	6.51	Med	42	1.64	478.2	343.4	37.55	p__Pseudomonadota	c__Gammaproteobacteria	o__Burkholderiales	f__Burkholderiaceae_D	g__UBA7377	s__UBA7377 sp002170395
MAG.102	56.85	0	Med	147	0.77	12.8	6.2	41.65	p__Pseudomonadota	c__Gammaproteobacteria	o__Comchoanobacterales	-	-	-
MAG.99	66.28	0	Med	9	0.88	213.5	153.2	39.07	p__Pseudomonadota	c__Gammaproteobacteria	o__Comchoanobacterales	f__Comchoanobacteraceae	g__Comchoanobacter	-
MAG.100	73.67	0.86	Med	122	1.14	66.5	13.7	39.91	p__Pseudomonadota	c__Gammaproteobacteria	o__Comchoanobacterales	f__UBA1515	g__UBA1515	s__UBA1515 sp002323935
MAG.101	81.98	0.29	Med	27	1.47	334.8	138.3	38.18	p__Pseudomonadota	c__Gammaproteobacteria	o__Comchoanobacterales	f__UBA7916	-	-
MAG.103	94.41	1.38	High	321	4.58	128.2	21.2	44.89	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	-	-
MAG.104	97.89	1.12	High	165	4.41	124.8	41.6	50.13	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	-	-
MAG.105	79.58	1.84	Med	121	3.12	171.9	47.2	42.92	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	-	-
MAG.106	97.34	1.69	High	88	3.80	290.3	74.5	45.81	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	g__Alteromonas_E	-
MAG.107	96.12	1.22	High	197	3.97	132.7	31.6	45.2	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Alteromonadaceae	g__Psychrosphaera	-
MAG.108	91.15	2.17	High	284	4.88	104.6	26.9	45.4	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Vibrionaceae	g__Vibrio	s__Vibrio coralliilyticus
MAG.109	59.25	9.48	Med	220	1.36	20.1	7.1	45.88	p__Pseudomonadota	c__Gammaproteobacteria	o__Enterobacterales_A	f__Vibrionaceae	g__Vibrio	s__Vibrio fortis_A
MAG.110	67.29	4.7	Med	112	0.99	51.0	13.3	34.26	p__Pseudomonadota	c__Gammaproteobacteria	o__GCA-002705445	f__GCA-002716945	g__CACLCV01	s__CACLCV01 sp913046125
MAG.111	93.22	3.79	High	198	4.61	125.4	41.7	49.82	p__Pseudomonadota	c__Gammaproteobacteria	o__Granulosicoccales	f__Granulosicoccaceae	-	-
MAG.112	83.73	2.53	Med	385	2.88	31.9	8.7	46.14	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Cellvibrionaceae	-	-
MAG.113	82.76	5.17	Med	190	2.94	78.2	24.0	43.66	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Cellvibrionaceae	-	-
MAG.114	60.82	0	Med	427	2.23	19.3	5.5	42.75	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Cellvibrionaceae	-	-
MAG.115	69.12	10.34	Med	694	3.58	25.0	5.6	42.94	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Cellvibrionaceae	g__Endobugula	-
MAG.116	92.67	1.41	High	138	2.30	115.9	25.4	46.09	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__DSM-6294	-	-
MAG.117	92.22	0	High	88	3.24	146.7	58.0	48.43	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__DT-91	g__DT-91	-
MAG.118	93.1	1.35	High	215	3.42	85.3	24.1	48.38	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Endozoicomonadaceae	-	-
MAG.119	96.9	2.44	High	281	5.14	82.1	30.1	47.29	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Endozoicomonadaceae	g__Endozoicomonas	-
MAG.120	93.39	5.71	Med	223	2.57	69.0	17.2	53.31	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Halieaceae	g__Luminiphilus	-

MAG ID	Comp. (%)	Cont.	Qual.	Contig num.	Genome size (Mbp)	Longest contig (kbp)	N50 (kbp)	GC (%)	Phylum	Class	Order	Family	Genus	Species
MAG.121	74.16	0.84	Med	246	1.80	57.8	8.3	53.58	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Halieaceae	g__Luminiphilus	-
MAG.122	91.23	2.03	High	168	2.58	78.3	21.5	57.61	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Halieaceae	g__Luminiphilus	s__Luminiphilus sp016778085
MAG.123	88.7	4.06	Med	234	2.44	39.3	15.1	57.84	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Halieaceae	g__Luminiphilus	s__Luminiphilus sp902520195
MAG.124	89.99	1.96	Med	243	3.66	106.8	25.8	64.73	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	-	-
MAG.125	95.98	2.9	High	161	4.32	113.4	47.5	64.52	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	-	-
MAG.126	49.61	4.85	Med	641	3.83	47.6	6.8	47.2	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	g__UBA4421	-
MAG.127	61.83	3.45	Med	230	2.21	67.4	12.5	55.03	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	g__UBA4421	-
MAG.128	91.87	1.53	High	50	3.15	330.2	109.5	52.61	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	g__UBA4421	s__UBA4421 sp002450255
MAG.129	91.73	0.79	High	28	2.64	308.0	153.7	56.22	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	g__UBA4421	s__UBA4421 sp002470275
MAG.130	74.06	2.25	Med	178	2.15	66.8	17.5	55.68	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	g__UBA4421	s__UBA4421 sp004213835
MAG.131	59.21	2.02	Med	329	1.86	33.4	6.1	52.93	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	g__UBA4421	s__UBA4421 sp902565895
MAG.132	87.53	3.35	Med	178	2.61	84.6	22.6	54.92	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__HTCC2089	g__UBA4421	s__UBA4421 sp902613475
MAG.133	77.92	0.62	Med	94	1.79	126.7	38.0	50.54	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Litoricolaceae	g__HIMB30	-
MAG.134	92.77	6.4	Med	84	1.90	172.3	61.2	50.08	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Litoricolaceae	g__HIMB30	s__HIMB30 sp002691485
MAG.135	73.7	4.43	Med	237	1.58	54.8	7.7	48.96	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Litoricolaceae	g__HIMB30	s__HIMB30 sp902558075
MAG.136	86.36	1.72	Med	177	3.39	77.9	35.0	42.35	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Marinomonadaceae	g__Marinomonas	-
MAG.137	56.18	0.41	Med	309	1.33	13.1	4.3	44.64	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Moraxellaceae	g__Psychrobacter	s__Psychrobacter pacificensis
MAG.138	93.54	1.39	High	48	3.58	306.6	137.4	42.48	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Natronospirillaceae	g__Reinekea	-
MAG.139	86.64	1.6	Med	74	1.86	198.7	53.4	38.35	p__Pseudomonadota	c__Gammaproteobacteria	o__Pseudomonadales	f__Porticoccaceae	g__AAA300-D14	s__AAA300-D14 sp002715065
MAG.140	57.41	5.04	Med	103	0.75	32.5	9.3	33.94	p__Pseudomonadota	c__Gammaproteobacteria	o__SAR86	f__AG-339-G14	g__AG-339-G14	s__AG-339-G14 sp003282105
MAG.141	64.83	6.37	Med	119	1.06	45.3	14.6	33.35	p__Pseudomonadota	c__Gammaproteobacteria	o__SAR86	f__AG-339-G14	g__AG-339-G14	s__AG-339-G14 sp902616715
MAG.142	52.43	4.95	Med	125	1.14	74.0	14.2	34.7	p__Pseudomonadota	c__Gammaproteobacteria	o__SAR86	f__D2472	g__CACKPV01	s__CACKPV01 sp902602185
MAG.143	49.76	1.67	Med	17	0.70	269.2	169.5	31.6	p__Pseudomonadota	c__Gammaproteobacteria	o__SAR86	f__D2472	g__MED-G85	s__MED-G85 sp003331505
MAG.144	50.47	8.01	Med	61	0.82	56.8	28.7	32.16	p__Pseudomonadota	c__Gammaproteobacteria	o__SAR86	f__D2472	g__SAR86A	s__SAR86A sp002690725
MAG.145	56.43	5.8	Med	89	0.55	23.7	7.3	34.66	p__Pseudomonadota	c__Gammaproteobacteria	o__SAR86	f__TMED112	g__TMED112	s__TMED112 sp902529055
MAG.146	72.03	3.13	Med	52	1.04	126.2	47.2	35.83	p__Pseudomonadota	c__Gammaproteobacteria	o__SAR86	f__TMED112	g__TMED112	s__TMED112 sp902535365
MAG.147	51.02	5.17	Med	77	0.47	33.9	6.8	33.7	p__Pseudomonadota	c__Gammaproteobacteria	o__SAR86	f__TMED112	g__TMED112	s__TMED112 sp902585315
MAG.148	56.33	8.31	Med	106	0.81	56.1	9.6	34.98	p__Pseudomonadota	c__Gammaproteobacteria	o__SAR86	f__TMED112	g__TMED112	s__TMED112 sp902610995
MAG.149	91.26	0.41	High	19	1.66	442.5	187.8	44.72	p__Pseudomonadota	c__Gammaproteobacteria	o__UBA4575	f__UBA4575	g__UBA1858	-
MAG.150	74.07	2.67	Med	540	3.77	43.1	8.6	46.15	p__SAR324	c__SAR324	o__SAR324	f__NAC60-12	g__JCVI-SCAAA005	s__JCVI-SCAAA005 sp002450295
MAG.151	100	0	High	55	1.97	133.7	61.1	52.34	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__DSM-45221	g__Coraliomargarita	s__Coraliomargarita sp002469895
MAG.152	82.09	1.35	Med	139	1.16	45.5	10.8	38.65	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__DSM-45221	g__GCA-2690565	-
MAG.153	97.3	0	High	54	1.33	109.5	49.8	38.47	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__DSM-45221	g__GCA-2690565	s__GCA-2690565 sp002457235
MAG.154	97.64	0.68	High	54	1.74	206.1	48.2	41.79	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__DSM-45221	g__GCA-2699365	s__GCA-2699365 sp022448585
MAG.155	64.58	3.04	Med	115	1.27	81.8	17.0	48.91	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__DSM-45221	g__UBA7441	s__UBA7441 sp002862945
MAG.156	63.09	7.09	Med	177	1.17	30.6	8.0	50.01	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__DSM-45221	g__UBA7441	s__UBA7441 sp902587165
MAG.157	97.26	1.01	High	70	2.50	184.1	64.6	44.35	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__MB11C04	g__MB11C04	-
MAG.158	86.79	2.7	Med	301	2.26	46.5	9.1	45.01	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__MB11C04	g__MB11C04	s__MB11C04 sp902615835
MAG.159	98.65	0	High	120	2.60	87.1	36.8	44.49	p__Verrucomicrobiota	c__Verrucomicrobiae	o__Opitutales	f__MB11C04	g__MB11C04	s__MB11C04 sp902618175

Table 3.2. Assembly statistics, completion matrices, and the taxonomic information for non-redundant metagenomic assembled genomes (MAGs). Assembly and completion matrices were generated using CheckM v1.2.2 , and the taxonomy was assigned with GTDB-Tk v2.3.0 using the Genome Taxonomy Database R214.

3.3.4 The abundance of phosphonate transport and degradation genes does not vary across samples

An investigation of the unbinned metagenomic co-assembly, constructed using high-quality paired reads from all samples, revealed no significant variation in the abundance of phosphonate transport and degradation genes across the three outside and three inside seawater samples (p-value ≥ 0.317; Figure 3.6). Gene abundance was quantified by calculating the transcripts per million (TPM) value for each specific gene. Please note that figure 3.6 shows a $\log_{10}$ transformed value of TPM for visual purposes. The minimal differences in gene abundance values can be attributed to the analysis being conducted at the sample level rather than the metagenome-assembled genome (MAG) level.

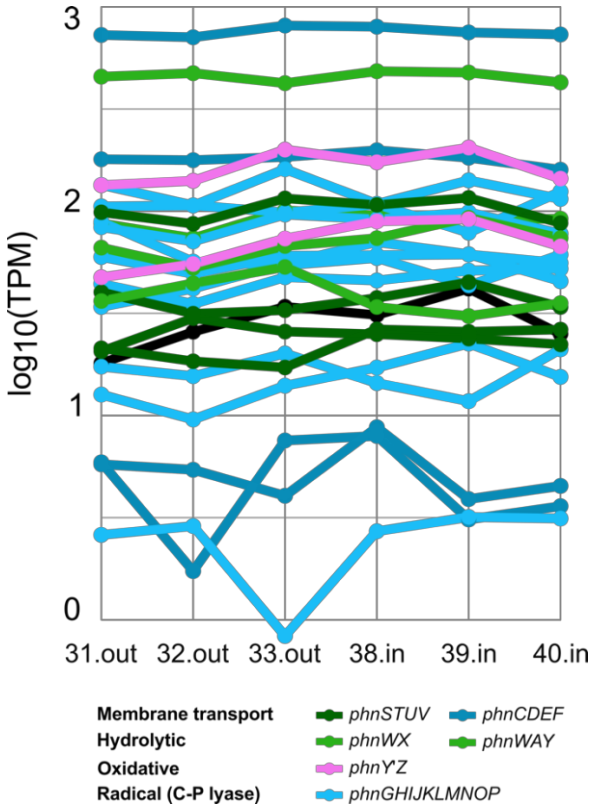


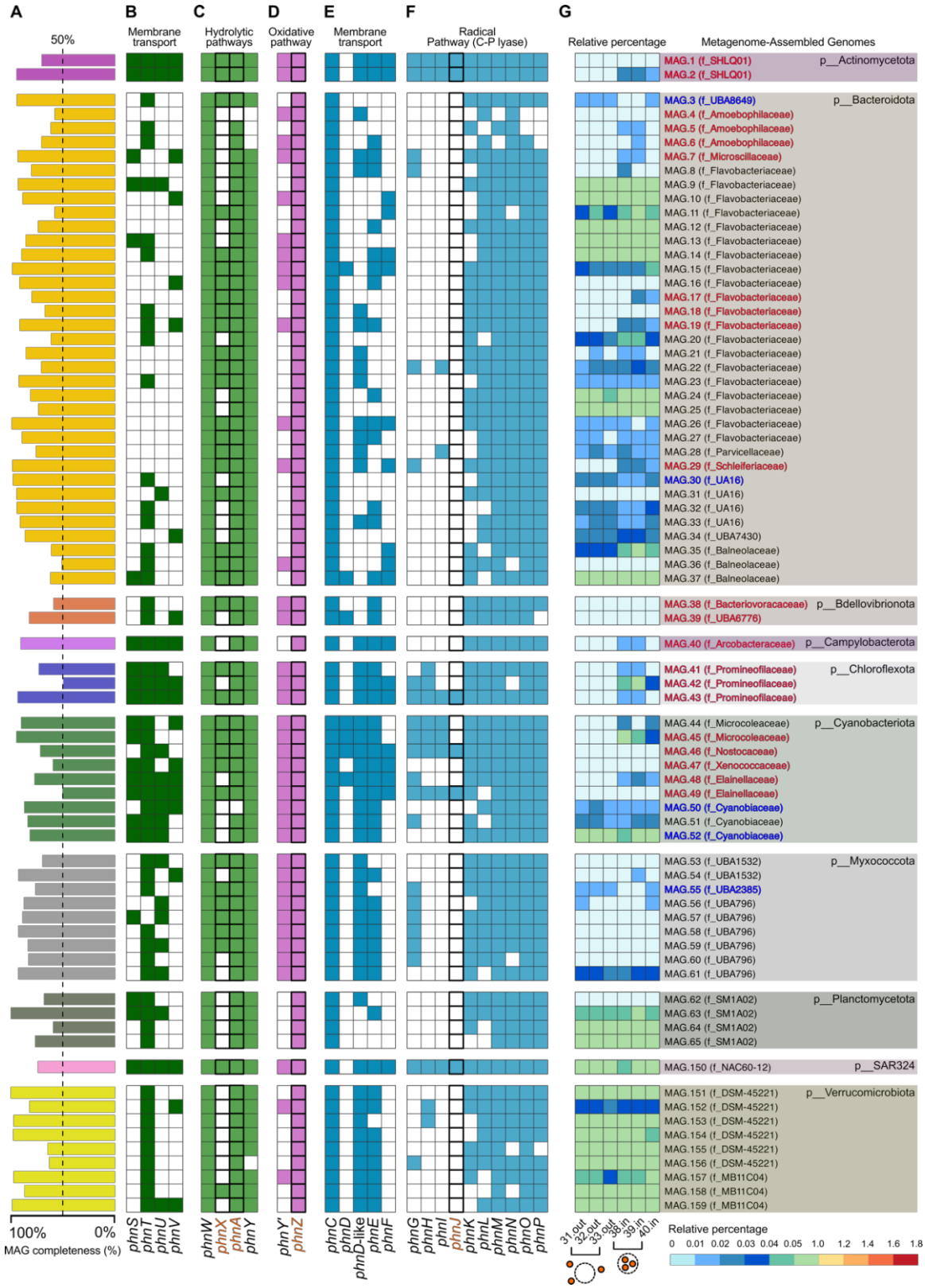
Figure 3.6. Parallel coordinate plot showing the abundance of phosphonate transport and degradation genes as $\log_{10}(1+\text{TPM})$. Each gene is colored coded by their function and known pathway assignments shown in Figure 3.1.

3.3.5 Phosphonate transport and degradation systems are redundant and widespread across bacterial MAGs belonging to multiple lineages

In addition to searching for phosphonate transport and degradation genes in our samples by mapping reads to the unbinned metagenomic assembly and calculating the TPM values for each gene per sample, we predicted and annotated these genes in our batch of medium- and high-quality MAGs (n=159). For the purpose of this study, a pathway is considered complete if all the associated genes are present in the MAGs under examination. Our overall results show that multiple bacterial lineages from diverse taxonomic clades possess the genetic machinery to transport and degrade phosphonates (Figure 3.7 & Table 3.3). This trend was not observed in two phyla—MAGs within *Campylobacterota* and *Planctomycetota* contained only one complete phosphonate degradation pathway, PhnWAY; whereas MAGs from all other phyla have the genetic potential to break down phosphonates using multiple mechanisms (Figure 3.7 & Table 3.3).

Moreover, a total of ~6% of our MAGs possessed all the required genes for metabolizing phosphonates via the PhnWX, PhnWAY, PhnY'Z, and C-P lyase pathways (Table 3.3). These representative bacteria also have all the transport genes that are part of the C-P lyase operon (i.e., *phnCDE*) and/or the PhnSTUV system. These nine MAGs span four phyla—*Actinomycetota*, *Chloroflexota*, *Pseudomonadota*, and *SAR324*. Such metabolic redundancy has been observed by others. For instance, an analysis of metagenomes from the Joint Genome Institute Integrated Microbial Genomes and Microbiomes (IMG/MER) and the Global Ocean Reference Genomes Tropics (GORG-Tropics) database revealed that *phnA* co-occurs with *phnX* in 6% to 12% of the organisms that contained phosphonate cycling genes (Lockwood et al., 2022). This same study also found that *phnZ* was linked to *phnA* or *phnX*

in 30% to 50% genomes if they had more than hydrolases for degrading phosphonates. This aligns with our observation of detecting *phnZ* in every MAG, except one, that have one or both of the phnWAY and the phnWX pathways. *PhnZ* is an iron-dependent oxygenase, and it possible that the availability of iron from the Sahara Desert dust clouds affords microbes of the Sargasso Sea the ability to retain and potential express this gene if necessary.


A
B
Membrane transport
C
Hydrolytic pathways
D
Oxidative pathway
E
Membrane transport
F
Radical Pathway (C-P lyase)
G
Relative percentage
Metagenome-Assembled Genomes
50%
100%
0%
MAG completeness (%)
phnS phnT phnU phnV
phnW phnX phnA phnY
phnY' phnZ
phnC phnD phnD-like phnE phnF
phnG phnH phnI phnJ phnK phnL phnM phnN phnO phnP
31.out 32.out 33.out 38.in 39.in 40.in
Relative percentage
0 0.01 0.02 0.03 0.04 0.05 1.0 1.2 1.4 1.6 1.8
MAG.1 (f_SHLQ01)
MAG.2 (f_SHLQ01)
p__Actinomycetota
MAG.3 (f_UBA8649)
MAG.4 (f_Amoebophilaceae)
MAG.5 (f_Amoebophilaceae)
MAG.6 (f_Amoebophilaceae)
MAG.7 (f_Microscillaceae)
MAG.8 (f_Flavobacteriaceae)
MAG.9 (f_Flavobacteriaceae)
MAG.10 (f_Flavobacteriaceae)
MAG.11 (f_Flavobacteriaceae)
MAG.12 (f_Flavobacteriaceae)
MAG.13 (f_Flavobacteriaceae)
MAG.14 (f_Flavobacteriaceae)
MAG.15 (f_Flavobacteriaceae)
MAG.16 (f_Flavobacteriaceae)
MAG.17 (f_Flavobacteriaceae)
MAG.18 (f_Flavobacteriaceae)
MAG.19 (f_Flavobacteriaceae)
MAG.20 (f_Flavobacteriaceae)
MAG.21 (f_Flavobacteriaceae)
MAG.22 (f_Flavobacteriaceae)
MAG.23 (f_Flavobacteriaceae)
MAG.24 (f_Flavobacteriaceae)
MAG.25 (f_Flavobacteriaceae)
MAG.26 (f_Flavobacteriaceae)
MAG.27 (f_Flavobacteriaceae)
MAG.28 (f_Parvicellaceae)
MAG.29 (f_Schleiferiaceae)
MAG.30 (f_UA16)
MAG.31 (f_UA16)
MAG.32 (f_UA16)
MAG.33 (f_UA16)
MAG.34 (f_UBA7430)
MAG.35 (f_Balneolaceae)
MAG.36 (f_Balneolaceae)
MAG.37 (f_Balneolaceae)
p__Bacteroidota
MAG.38 (f_Bacteriovoracaceae)
MAG.39 (f_UBA6776)
p__Bdellovibrionota
MAG.40 (f_Arcobacteraceae)
p__Campylobacterota
MAG.41 (f_Promineofilaceae)
MAG.42 (f_Promineofilaceae)
MAG.43 (f_Promineofilaceae)
p__Chloroflexota
MAG.44 (f_Microcoleaceae)
MAG.45 (f_Microcoleaceae)
MAG.46 (f_Nostocaceae)
MAG.47 (f_Xenococcaceae)
MAG.48 (f_Elainellaceae)
MAG.49 (f_Elainellaceae)
MAG.50 (f_Cyanobiaceae)
MAG.51 (f_Cyanobiaceae)
MAG.52 (f_Cyanobiaceae)
p__Cyanobacteriota
MAG.53 (f_UBA1532)
MAG.54 (f_UBA1532)
MAG.55 (f_UBA2385)
MAG.56 (f_UBA796)
MAG.57 (f_UBA796)
MAG.58 (f_UBA796)
MAG.59 (f_UBA796)
MAG.60 (f_UBA796)
MAG.61 (f_UBA796)
p__Myxococcota
MAG.62 (f_SM1A02)
MAG.63 (f_SM1A02)
MAG.64 (f_SM1A02)
MAG.65 (f_SM1A02)
p__Planctomycetota
MAG.150 (f_NAC60-12)
p__SAR324
MAG.151 (f_DSM-45221)
MAG.152 (f_DSM-45221)
MAG.153 (f_DSM-45221)
MAG.154 (f_DSM-45221)
MAG.155 (f_DSM-45221)
MAG.156 (f_DSM-45221)
MAG.157 (f_MB11C04)
MAG.158 (f_MB11C04)
MAG.159 (f_MB11C04)
p__Verrucomicrobiota

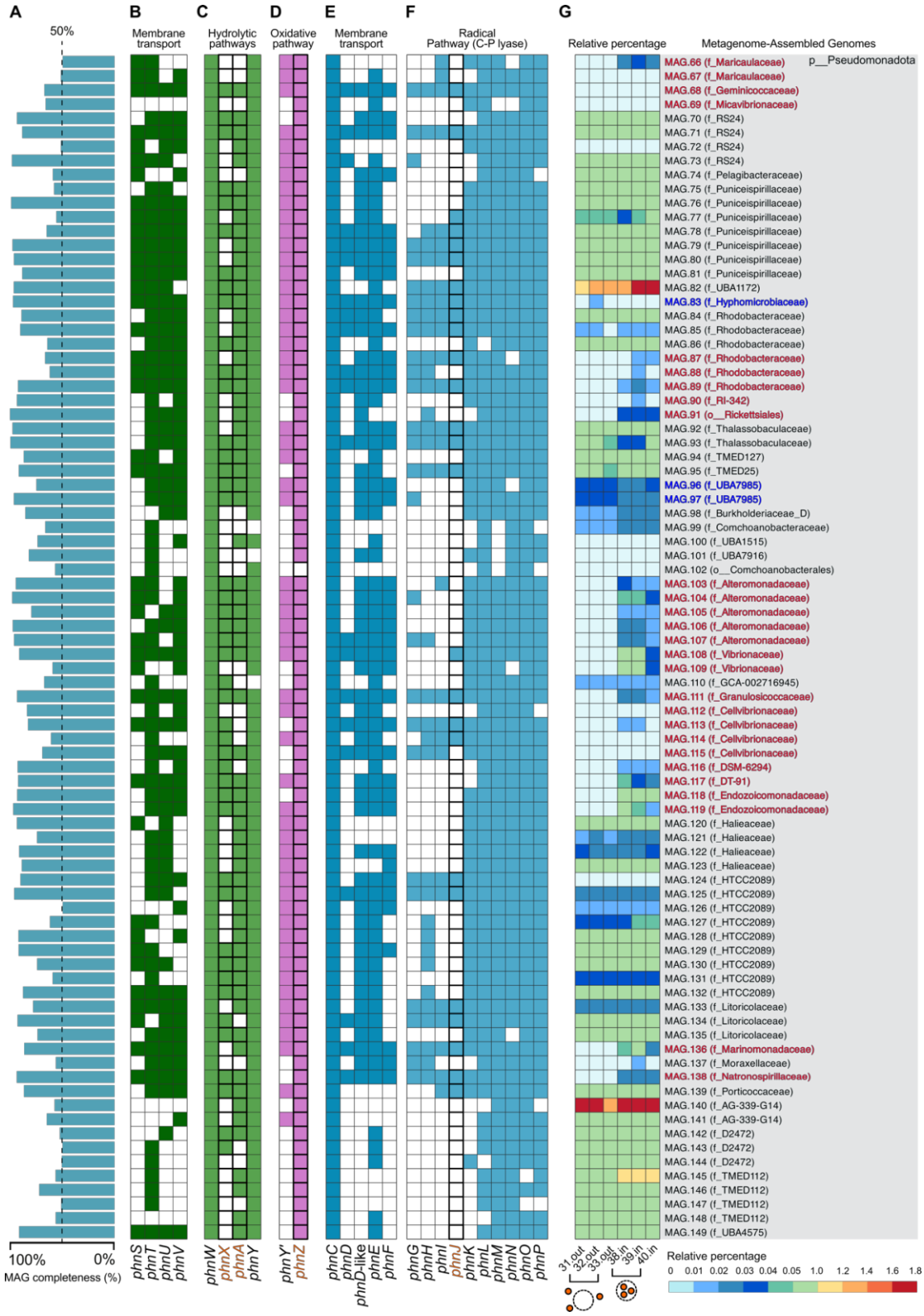

A
50%
100%
0%
MAG completeness (%)
B
Membrane transport
phnS
phnT
phnU
phnV
C
Hydrolytic pathways
phnW
phnX
phnA
phnY
D
Oxidative pathway
phnY'
phnZ
E
Membrane transport
phnC
phnD
phnD-like
phnE
phnF
F
Radical Pathway (C-P lyase)
phnG
phnH
phnI
phnJ
phnK
phnL
phnM
phnN
phnO
phnP
G
Relative percentage
Metagenome-Assembled Genomes
p__Pseudomonadota
MAG.66 (f_Maricaulaceae)
MAG.67 (f_Maricaulaceae)
MAG.68 (f_Geminicoccaceae)
MAG.69 (f_Micavibrionaceae)
MAG.70 (f_RS24)
MAG.71 (f_RS24)
MAG.72 (f_RS24)
MAG.73 (f_RS24)
MAG.74 (f_Pelagibacteraceae)
MAG.75 (f_Puniceispirillaceae)
MAG.76 (f_Puniceispirillaceae)
MAG.77 (f_Puniceispirillaceae)
MAG.78 (f_Puniceispirillaceae)
MAG.79 (f_Puniceispirillaceae)
MAG.80 (f_Puniceispirillaceae)
MAG.81 (f_Puniceispirillaceae)
MAG.82 (f_UBA1172)
MAG.83 (f_Hyphomicrobiaceae)
MAG.84 (f_Rhodobacteraceae)
MAG.85 (f_Rhodobacteraceae)
MAG.86 (f_Rhodobacteraceae)
MAG.87 (f_Rhodobacteraceae)
MAG.88 (f_Rhodobacteraceae)
MAG.89 (f_Rhodobacteraceae)
MAG.90 (f_RI-342)
MAG.91 (o__Rickettsiales)
MAG.92 (f_Thalassobaculaceae)
MAG.93 (f_Thalassobaculaceae)
MAG.94 (f_TMED127)
MAG.95 (f_TMED25)
MAG.96 (f_UBA7985)
MAG.97 (f_UBA7985)
MAG.98 (f_Burkholderiaceae_D)
MAG.99 (f_Comchoanobacteraceae)
MAG.100 (f_UBA1515)
MAG.101 (f_UBA7916)
MAG.102 (o__Comchoanobacterales)
MAG.103 (f_Alteromonadaceae)
MAG.104 (f_Alteromonadaceae)
MAG.105 (f_Alteromonadaceae)
MAG.106 (f_Alteromonadaceae)
MAG.107 (f_Alteromonadaceae)
MAG.108 (f_Vibrionaceae)
MAG.109 (f_Vibrionaceae)
MAG.110 (f_GCA-002716945)
MAG.111 (f_Granulosicoccaceae)
MAG.112 (f_Cellvibrionaceae)
MAG.113 (f_Cellvibrionaceae)
MAG.114 (f_Cellvibrionaceae)
MAG.115 (f_Cellvibrionaceae)
MAG.116 (f_DSM-6294)
MAG.117 (f_DT-91)
MAG.118 (f_Endozoicomonadaceae)
MAG.119 (f_Endozoicomonadaceae)
MAG.120 (f_Halieaceae)
MAG.121 (f_Halieaceae)
MAG.122 (f_Halieaceae)
MAG.123 (f_Halieaceae)
MAG.124 (f_HTCC2089)
MAG.125 (f_HTCC2089)
MAG.126 (f_HTCC2089)
MAG.127 (f_HTCC2089)
MAG.128 (f_HTCC2089)
MAG.129 (f_HTCC2089)
MAG.130 (f_HTCC2089)
MAG.131 (f_HTCC2089)
MAG.132 (f_HTCC2089)
MAG.133 (f_Litoricolaceae)
MAG.134 (f_Litoricolaceae)
MAG.135 (f_Litoricolaceae)
MAG.136 (f_Marinomonadaceae)
MAG.137 (f_Moraxellaceae)
MAG.138 (f_Natronospirillaceae)
MAG.139 (f_Porticoccaceae)
MAG.140 (f_AG-339-G14)
MAG.141 (f_AG-339-G14)
MAG.142 (f_D2472)
MAG.143 (f_D2472)
MAG.144 (f_D2472)
MAG.145 (f_TMED112)
MAG.146 (f_TMED112)
MAG.147 (f_TMED112)
MAG.148 (f_TMED112)
MAG.149 (f_UBA4575)
31 out
32 out
33 out
38 in
39 in
40 in
Relative percentage
0 0.01 0.02 0.03 0.04 0.05 1.0 1.2 1.4 1.6 1.8

Figure 3.7. (A) Bar plot depicting the percent completeness of the metagenomic assembled genomes. The dotted line is the 50% threshold. (B-F) Heatmaps with presence (filled) and absence (unfilled) data for phosphonate transport and degradation genes from figure 3.1 (from left to right: *phnSTUV* (transport), *phnWX* and *phnWAY*, *phnY'Z*, *phnCDEF* (transport), and *phnGHIJKLMNOP*). The pathway types are presented above the heatmaps, and the genes in orange text encode the catalytic enzymes that are responsible for cleaving the C-P bond of phosphonates. The squares showing the presence and absence data of these genes are outlined with thicker black borders compare to other genes. We also show the presence and absence of an additional gene, *phnD*-like. (G) Heatmap showing the relative percentages of all 159 nonredundant MAGs. MAGs with a statistically significant log fold change of at least 1.5 inside the patch compared to outside the patch are shown in red text, and the MAGs labeled in blue text that have a log fold change -1.5 or less are more abundant outside the patch.

	Avg. comp. (%)	Num of MAGs	phnWX	phnWAY	phnY'Z	C-P lyase
p__Actinomycetota	82.43	2	2	2	2	2
p__Bacteroidota	81.17	35	19	32	12	0
p__Bdellovibrionota	70.67	2	1	2	2	0
p__Campylobacterota	90.53	1	0	1	0	0
p__Chloroflexota	72.10	3	2	3	3	1
p__Cyanobacteriota	77.39	9	5	8	9	1
p__Myxococcota	85.23	9	6	9	9	0
p__Planctomycetota	76.14	4	0	4	0	0
p__Pseudomonadota	78.71	84	44	65	57	15
p__SAR324	74.07	1	1	1	1	1
p__Verrucomicrobiota	87.49	9	1	8	2	0
Total	-	159	81	135	97	20

Table 3.3. Presented are the average genome completion percentages and the number of MAGs containing complete phosphonate degradation pathways for each of the 11 phyla.

	Avg. comp. (%)	Num of MAGs	*phnX*	*phnA*	*phnZ*	*phnJ*
p__Actinomycetota	82.43	2	2	2	2	2
p__Bacteroidota	81.17	35	19	**34**	**35**	0
p__Bdellovibrionota	70.67	2	1	2	2	0
p__Campylobacterota	90.53	1	0	1	**1**	0
p__Chloroflexota	72.10	3	2	3	3	1
p__Cyanobacteriota	77.39	9	5	8	9	**2**
p__Myxococcota	85.23	9	6	9	9	0
p__Planctomycetota	76.14	4	0	4	**4**	0
p__Pseudomonadota	78.71	84	44	**69**	**83**	**26**
p__SAR324	74.07	1	1	1	1	1
p__Verrucomicrobiota	87.49	9	1	**9**	**9**	0
Total	-	159	81	**142**	**158**	**32**

Table 3.4. Presented are the average genome completion percentages and the number of MAGs, from each of the 11 phyla, containing the genes that encode the catalytic enzyme responsible for cleaving the C-P bond. The number in orange text differ from the count data presented in table 3.3.

3.3.6 Substrate-specific pathways are more common than the broad-range C-P lyase pathway

We identified a higher prevalence of substrate-specific pathways compared to the broad-range C-P lyase. Of the 159 genomes, 56 representative bacteria have all three substrate-specific pathways, and between 58 and 88 MAGs contained at least one of the three substrate-specific pathways (Figure 3.1). The presence of more substrate-specific pathways may be attributed to the fact that these pathways are driven by enzymes encoded by a limited number of genes, or more likely, due to the high concentrations of the substrate in the seawater (*e.g.*, 2-AEP). For instance, 2-AEP is indeed a widely distributed phosphonate that occurs naturally, especially in marine environments (Hilderbrand, 2018).

In terms of the most common substrate-specific pathway among our MAGs, the hydrolytic PhnWAY mechanism was detected in 135 out of 150 MAGs (85%) followed by PhnY'Z (61%), PhnWX (51%), and C-P lyase (14%). PhnWAY being the most abundant pathway in our representative genomes is not consistent with previous work. For example, Martinez and coworkers' pioneering work from 2010 showed that a higher percent of microorganisms contained *phnJ* of C-P lyase (28%) than *phnA* (10%) in the Sargasso Sea (A. Martinez et al., 2010). Similarly, a recent study found *phnJ* to be enriched in the epipelagic zones of the North Atlantic Ocean and the Mediterranean Sea, two P-deplete regions (Sosa et al., 2019). Lastly, a study undertaken by Lockwood and colleagues also provided supporting evidence from surveys of TARA Oceans metagenomes that *phnZ* is more common in the surface ocean waters than *phnA* (Lockwood et al., 2022).

The above discrepancy between our study and other works could be due to a variety of reasons. The findings from previous literature cited here are based on surveys of

metagenomic data on a global scale, whereas our study focuses on free-living bacteria and their phosphonate transport and degradation abilities within an ecosystem rich with organic matter—*Sargassum* patches. Furthermore, we denote a pathway as complete if all genes are present in the genomes; however, many conclusions in previous works were formed based prevalence of individual *phn* genes. This could also result in different findings as we see here. To explore this possibility, we calculated the percentage of MAGs containing only the genes encoding for the catalytic enzymes (*i.e.*, *phnX*, *phnA*, *phnZ*, and *phnJ*). We found that 158 out of 159 MAGs (99%) have the potential to degrade phosphonates via the oxidative pathway involving *phnZ*, as compared to the 61% of MAGs when considering all the genes in this pathway (Table 3.4). When focusing exclusively on the catalytic genes, our findings corroborate the previous results by Lockwood and colleagues, who identified PhnZ as the primary mechanism for phosphonate degradation among marine bacteria. However, our results remain divergent from the study conducted by Martinez et al. (2010). This discrepancy may be attributed to differences in sampling depths. Specifically, our samples were collected from surface ocean waters, whereas Martinez and colleagues collected samples from depths of 25 m or greater. Additionally, the deviation may stem from the fact that Martinez et al. (2010) were among the first to identify the *PhnY'Z* gene sequences. Since then, the improvement and expansion of genomic databases have greatly enhanced the detection and classification of organisms possessing the *phnY'Z* genes, thereby increasing our understanding and knowledge of their distribution.

3.3.7 Members of the *Pseudomonadales* order are more prevalent inside *Sargassum* patch and contain at least one phosphonate degradation pathway

In general, members of the *Alphaproteobacteria* and *Gammaproteobacteria* classes (*Pseudomonadota* phylum) were well-represented in our MAGs (Figure 3.8). These findings agree with past studies which also found these classes to be more prevalent in the North Atlantic Ocean and other P-limited marine habitats (Lockwood et al., 2022; A. Martinez et al., 2010; Sosa et al., 2019). Furthermore, the ASVs of the *Pseudomonadota* phylum were prevalent in our 16S rRNA data. Therefore, building on our findings based on the 16S rRNA data, we next identified MAGs with significantly greater relative abundance inside the *Sargassum* patch versus outside the patch. Using a fold change threshold of at least 1.5x, we determined that 48 MAGs exceeded this threshold inside the patch while only 8 surpassed the minimum fold change outside the patch (Figure 3.8H). All 56 MAGs spanned 8 out of the 11 phyla represented in our metagenomic dataset— *Pseudomonadota*, *Bacteroidota*, *Cyanobacteriota*, *Chloroflexota*, *Actinomycetota*, *Bdellovibrionota*, *Campylobacterota*, and *Myxococcota*. Members from the phyla *Planctomycetota*, *SAR324*, and *Verrucomicrobiota* were equally abundant inside and outside the patch.

Among the 8 phyla with MAGs that were present at a fold change of 1.5x or greater inside the patch, approximately 83% of them belonged to *Pseudomonadota* (~56%), *Bacteroidota* (~17%), and *Cyanobacteriota* (10%). Within *Pseudomonadota*, families which were consistently more pervasive inside the patch across the samples were f_*Maricaulaceae*, f_*Rhodobacteraceae*, o_*Rickettsiales* (family not determined), f_*Alteromonadaceae*, f_*Vibrionaceae*, f_*Granulosicoccaceae*, f_*DSM-6294*, f_*DT-91*, f_*Endozoicomonadaceae*, f_*Marinomonadaceae*, and f_*Natronospirillaceae*. Some microbial constituents of these

families had all genes involved the phosphonate uptake and transport and degradation, while some had incomplete sets of genes. For instance, a *Rhodobacteraceae* genome (MAG.89) possessed all the genes, but its relatives in the same family were missing genes encoding the catalytic enzymes (Figure 3.8A-F). Similarly, all MAGs from the *Alteromonadaceae* family contained all the genes involved in the two hydrolytic (PhnWX and PhnWAY) and one oxidative pathway (PhnY'Z) but were lacking several genes from PhnSTUV and the C-P lyase systems. In general, all significantly abundant MAGs inside the patch from phylum *Pseudomonadota* contained at least one phosphonate degradation pathways except for MAG.69 (family *Micavibrionaceae*) and MAG.109 (genus *Vibrio*) (Figure 3.8A-F) and 22 MAGs from this phylum out of a total of 27 have the potential to uptake and transport phosphonates via the PhnSTUV and/or the PhnCDE systems (Figure 3.8A-F). Members of the family *Micavibrionaceae* are close relatives of predatory bacteria with streamlined genomes that lack many biosynthetic genes (Pasternak et al., 2014; Wang et al., 2011), which could explain the absence of phosphonate degradation genes in our *Micavibrionaceae* MAG.

Considering the differentially abundant ASVs inside our *Sargassum* patch samples, we also searched for MAGs that could be represented by these amplicon variants. Within the *Pseudomonadota* phylum, we classified two MAGs that matched the top two genera from the same phylum in our 16S rRNA data which were present across all three inside patch samples. MAG.136 (genus *Marinomonas*) shared taxonomic identifications with ASV58 and ASV80 (genus *Marinomonas*) and MAG.138 (genus *Reinekea*) is a representative genome of ASV11 and ASV83 (genus *Reinekea*). Both genera are comprised of motile heterotrophic bacteria that are strictly aerobic or facultative anaerobic (Espinosa et al., 2010; H. Kang et al., 2016). *Marinomonas* members can be found in a variety of marine environments including

seagrasses and other brown algal species (Espinosa et al., 2010; Martin et al., 2015). A *Reinekea* representative was first isolated from marine sediments and the genus contains a few species which are all marine in origin (H. Kang et al., 2016; Romanenko et al., 2004).

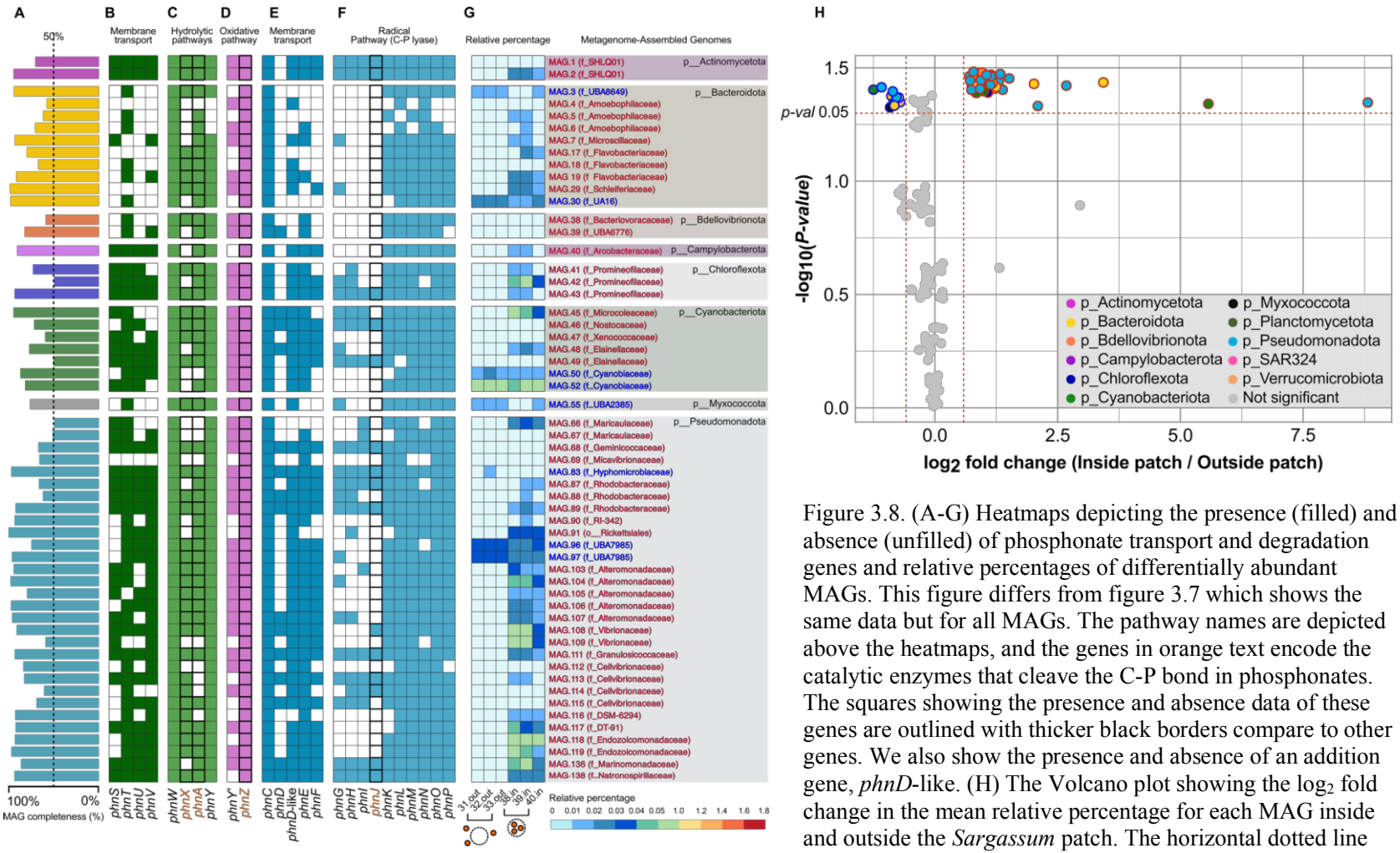


Figure 3.8. (A-G) Heatmaps depicting the presence (filled) and absence (unfilled) of phosphonate transport and degradation genes and relative percentages of differentially abundant MAGs. This figure differs from figure 3.7 which shows the same data but for all MAGs. The pathway names are depicted above the heatmaps, and the genes in orange text encode the catalytic enzymes that cleave the C-P bond in phosphonates. The squares showing the presence and absence data of these genes are outlined with thicker black borders compare to other genes. We also show the presence and absence of an addition gene, *phnD*-like. (H) The Volcano plot showing the $\log_2$ fold change in the mean relative percentage for each MAG inside and outside the *Sargassum* patch. The horizontal dotted line shows the p-value threshold of 0.05 while the two vertical dotted lines represent the 1.5-fold change thresholds in either direction. The samples are color coded based on phylum. (A-H) Circles and MAG IDs in red and blue show statistically significant MAGs inside and outside the patch, respectively.

3.3.8 *Sargassum* patch is enriched with alginate and fucoidan degrading bacteria

Pelagic *Sargassum* is a photosynthetic brown macroalga with a robust, carbohydrate-rich cell wall similar to that found in terrestrial plants. However, the polysaccharide constituents of brown macroalgal cell walls differ significantly from that of plants, with alginates and fucoidans being dominant components, including in *Sargassum* (Ortega-Flores et al., 2022). Microorganisms which synthesize enzymes for metabolizing complex polysaccharides, such as alginates and fucoidans, play a crucial role in the remineralization of carbon in marine environments. These specialized catalysts, known as carbohydrate active enzymes (CAZymes), form a large and diverse class of enzymes involved in the breakdown and modification of complex polysaccharides into smaller polymers and monomers (Gavande et al., 2023). Consequently, we examined the genomes of MAGs that were differentially abundant outside and inside the *Sargassum* patch for specific CAZymes that bacteria utilize to degrade alginates, fucoidans, and lignin. Although lignin is not a polysaccharide and brown macroalgal cell walls do not contain this compound per se (Dobrowolski et al., 2022), lignin-like polyphenolic compounds have been detected in pelagic *Sargassum* tissue samples in the Mexican Caribbean (Alzate-Gaviria et al., 2021) as well as in other *Sargassum* species (Ardalan et al., 2018). Therefore, lignin-degradation genes were included in our analysis.

Of the three types of compounds, our findings revealed that CAZymes that degrade lignin are more widespread in the free-living microbes from inside the *Sargassum* patch (Figure 3.9). For example, all MAGs except two, MAG.90 and MAG.141 (families *RI-342* and *AG-339-G14*, phylum *Pseudomonadota*), contained at least one out of the eight lignin catabolizing CAZymes that we analyzed. Furthermore, all MAGs that were more abundant inside the patch possessed multiple copies of these CAZymes. In particular, several gene

copies encoding enzymes of the Auxiliary Activity Family 5 (AA5) were present in our MAGs. This family is comprised of copper radical oxidases that use oxygen or reactive oxygen species to cleave the aromatic rings (Janusz et al., 2017).

Alginate lyase genes were the second most abundant CAZymes represented among our MAGs which spanned all phyla except *Bdellovibrionota* and *SAR324* (Figure 3.9). However, not many MAGs, that were more abundant inside the patch, contained several copies of the alginate-degrading genes. Two notable exceptions were MAG.136 (family *Marinomonadaceae*) and MAG.138 (family *Natronospirillaceae*), which matched the top two genera from the same phylum in our 16S rRNA data that were present across all three inside patch samples (Figures 3.4 & 3.9). As stated previously, MAG.136 (genus *Marinomonas*) shared taxonomic identifications with ASV58 and ASV80 (genus *Marinomonas*) and MAG.138 (genus *Reinekea*) is a representative genome of ASV11 and ASV83 (genus *Reinekea*). Moreover, 75% (n=8) of genomes that were more outside the patch lacked the genetic capacity for alginate degradation. These results suggest that alginate lyase genes are widespread among MAGs across various phyla, but may be more concentrated in specific taxa, such as *Marinomonas* and *Reinekea*, which are more abundant inside the patch. In contrast, the genetic capability to degrade fucoidan was rarer amongst our MAGs. For example, only 24% of our genomes had the CAZymes known to target this complex sulfated polysaccharide (Figure 3.9). Additionally, analogous to our findings pertaining to alginate, 60% and 38% of the MAGs present at higher relative proportions inside and outside the *Sargassum* patch, respectively, had no fucoidan degrading genes.

The above findings are significant, considering that pelagic *Sargassum* likely serves as an important source of complex carbohydrates and polyphenols. This is particularly

critical in the subtropical north Atlantic Ocean, where *Sargassum* patches are often the only physical structures available in the open ocean, providing vital ecological services. Furthermore, although it is challenging to form conclusions regarding gene expression and function of these CAZymes-encoding genes, their presence in our MAGs suggests that the free-living microbes within *Sargassum* patches have the metabolic potential to utilize *Sargassum*-derived carbohydrates and polyphenols as a carbon source. Culture-based and functional studies are necessary to fully comprehend if microbes living in close contact with *Sargassum* can indeed use these compounds to fuel their growth and production.

Figure 3.9. Heatmaps portraying the gene count data for alginate, fucoidan, and lignin degradation carbohydrate active enzymes (CAZymes). The specific family codes are provided at the bottom of the heatmaps. MAGs with a statistically significant log fold change of at least 1.5 inside the patch compared to outside the patch are shown in red text, and the MAGs labeled in blue text that have a log fold change -1.5 or less are more abundant outside the patch.

3.4. Conclusion

This investigation into the free-living microbial communities found within and outside *Sargassum* patches has uncovered important insights into their metabolic potential for phosphonate transport and catabolism. Our results revealed no significant differences in the abundance of phosphonate transport and degradation genes in microbes living inside versus outside *Sargassum* patches. This lack of variation may be attributed to the phosphorus-limited conditions of the subtropical north Atlantic Ocean, where the ability to break down phosphonates in the dissolved organic phosphorus pool is so crucial for growth and production that most microbes have evolved to have genetic mechanisms for transporting and catabolizing phosphonates.

Our work also demonstrates that specific carbohydrate active enzymes (CAZymes), especially those involved in lignin degradation, are more ubiquitous in the genomes of microbes living inside *Sargassum* patches. Although brown macroalgal cell walls do not contain lignin, lignin-like polyphenolic compounds have been detected in pelagic *Sargassum* tissue samples by other researchers. Furthermore, we found that alginate-catabolizing CAZymes are also well represented among these microbes, whereas fucoidan-degrading genes are less common. *Sargassum* cells walls are rich in lignin-like polyphenols, alginates, and fucoidans, and the potential ability to break down these compounds suggests that microbes living in *Sargassum* patches are well-adapted to utilize *Sargassum*-derived carbon sources.

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Appendix

I. The cetyltrimethylammonium bromide (CTAB) DNA extraction protocol

TLBE (Tris Lysis Buffer with Extra EDTA):

NaCl	100 mM
Tris HCl, pH 8	10 mM
EDTA, pH 8	100 mM
SDS	0.5% (w/v)

HS-CTAB Extraction Solution (High-Salt)

CTAB	2% (w/v)
Tris Cl pH 8	100 mM
EDTA	100 mM
NaCl	1.85 M

CTAB Precipitation Solution (2x)

CTAB	1% (w/v)
Tris Cl pH 8	50 mM
EDTA	100 mM

EB ("Elution Buffer"):

Tris HCl, pH 8	10 mM

1. Before beginning extraction, freshly prepare a 100 mg mL^{-1} solution of lysozyme.
2. Take tissue sample out of freezer and grind it down to a semi-powdery consistency.
3. Transfer the ground-up sample into a falcon tube.
4. Immediately add 500 µL of TLBE lysis buffer to the sample.
5. Add 50 µL of lysozyme.
6. Vortex quickly and thoroughly. Repeated short vortexing avoids wasting material on the side of tubes. NOTE: Avoid vortexing the sample after this step in order to prevent the high molecular weight DNA from degrading.
7. Incubate at 37°C for at least 1 hour (recommended speed 400 rpm for 5 sec followed by 10 min of rest).
8. Add three times the sample volume of warm HS-CTAB extraction buffer and 37.5 µL of Proteinase K (~20 mg mL^{-1}; NEB cat. no. P8107S).
9. Mix gently by inverting slowly (at least three times).
10. Incubate at 55°C for 3 hours, with mixing at 300 rpm for 5 sec every 10 min.
11. After incubation, cool to room temperature.
12. Next, add one times the sample volume of chloroform:isoamyl alcohol (24:1).

13. Mix for 20 minutes on rotary mixer at 30-40 rpm until a fine emulsion forms. If no emulsion, slowly increase speed and continue mixing.
14. Spin at 5000 g for 15 minutes (use a centrifuge at 4°C if possible).
15. Remove aqueous (top) layer to a fresh 2 mL Eppendorf tube.
16. Add 1/10 the sample volume of CTAB extraction buffer and one times the sample volume of chloroform:isoamyl alcohol (24:1) to the clean aqueous layer and extract again as in steps 13 – 15.
17. Remove aqueous layer to clean 2 mL Eppendorf (~500 µL).
18. Add two times the sample volume of warm CTAB precipitation buffer.
19. Mix gently by inversion.
20. Incubate overnight at 55°C in a shaking incubator at a gentle speed of ~300 rpm
21. After incubation, spin at 16000 g for 3 minutes at room temperature.
22. Remove supernatant.
23. Add 1 mL cold (-20°C) 80% ethanol to your pellet. Work pellet away from the side of the tube with either a gentle flick or by gentle agitating with a pipet tip. The pellet should be fully submerged in ethanol.
24. Incubate for 15 minutes.
25. After incubation, spin for 3 min at 16,000 g.
26. Remove ethanol.
27. RNase treat your samples at this step if desired.
28. Wash in 1 mL 80% ethanol again. Spin. Remove supernatant.
29. Air dry the pellet.
30. Resuspend in 30 µL EB; take care to get all the precipitate on tube sides.

31. Allow to rehydrate 1-2 days at 4°C.

32. Check the DNA quality and quantity using NanoDrop and Qubit or Bioanalyzer.

II. Accession identification numbers and taxonomic information for the 20 bacterial references used to detect amphibactin NRPSs

Species	Accesion	Assembly	NRPS region coordinates	Phylum	Class	Order	Family	Genus
Nitrosococcus halophilus Nc 4	NC_013960.1	GCF_000024725.1	1796409..1855079	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Nitrosococcus
Nitrosococcus wardiae	NZ_CP038033.1	GCF_004421105.1	2558420..2617089	Proteobacteria	Gammaproteobacteria	Chromatiales	Chromatiaceae	Nitrosococcus
Alcanivorax borkumensis SK2	NC_008260.1	GCF_000009365.1	2358710..2419758	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Alcanivoracaceae	Alcanivorax
Marinomonas arctica	NZ_CP061081.1	GCF_014623465.1	82494..143297	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Marinomonas
Marinomonas spartinae	NZ_FLOB01000001.1	GCF_900089775.1	592543..653425	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Marinomonas
Azotobacter beijerinckii	NZ_FOFJ01000014.1	GCF_900110885.1	50237..103821	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Azotobacter
Azotobacter chroococcum	NZ_CP011835.1	GCF_002220155.1	4319300..4380353	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Azotobacter
Pseudomonas alcaligenes	NZ_CP014784.1	GCF_001597285.1	2656307..2717313	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Pseudomonas guryensis	NZ_JACJFN010000002.1	GCF_014164785.1	775409..836310	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Pseudomonas ullengensis	NZ_JACJUD010000004.1	GCF_014174475.1	196385..257441	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
Vibrio bathopelagicus	NZ_CP062500.1	GCF_014879975.1	1895327..1956646	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
Vibrio coralliilyticus	NZ_CP063051.1	GCF_000967465.2	1402662..1463652	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
Vibrio cyclitrophicus	NZ_CP039700.1	GCF_005144905.1	1753102..1814328	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
Vibrio gigantis	NZ_CP092384.1	GCF_022371215.1	1612122..1673435	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
Vibrio neptunius	NZ_JXXU01000001.1	GCF_000967495.1	175441..236481	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
Vibrio ostreicida	NZ_MPHM01000004.1	GCF_001957165.1	66206..126954	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
Vibrio proteolyticus NBRC 13287	NZ_BATJ01000001.1	GCF_000467125.1	373793..434544	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
Vibrio bivalvicida	NZ_LLEI02000021.1	GCF_001399455.2	615480..676499	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio oreintalis group
Vibrio europaeus	NZ_LUAX01000007.1	GCF_001695575.1	1433131..1494159	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio oreintalis group
Vibrio tubiashii ATCC 19109	NZ_CP009355.1	GCF_000772105.1	690638..751633	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio oreintalis group