

EXPLORING THE BACTERIA-DIATOM METAORGANISM USING SINGLE-CELL WHOLE  
GENOME AMPLIFICATION

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## ABSTRACT

Diatoms are responsible for a large fraction of oceanic and freshwater biomass production and are critically important to sequestration of carbon to the deep ocean. As with most surfaces present in aquatic systems, bacteria colonize the exterior of living diatom cells, and interact with the diatom and each other. The health, success and productivity of diatoms may be better understood by considering them as metaorganisms composed of a host cell together with its attached bacterial assemblage. There is ample evidence that this diatom-associated bacterial assemblage is very different from free-living bacteria, but its composition, functional capabilities and impact on diatom health and productivity are poorly understood. In this study, I examined the relationship between diatoms and bacteria at the single-cell level. Samples were collected in a nutrient-limited system (Station ALOHA, 22° 45'N, 158° 00'W) at the deep chlorophyll maximum. Flow cytometry followed by multiple displacement amplification was used to isolate and investigate the bacterial assemblages attached to 40 individual host cells. Thirty-four host cells were diatoms, including 27 *Thalassiosira* spp., 3 *Chaetoceros* spp., and one each of *Pseudo-nitzschia* sp., *Guinardia* sp., *Leptocylindrus* sp., and *Delphineis* sp. The remaining host cells included dinoflagellates, coccolithophorids, and flagellates. The bacteria associated with each host were identified by amplifying, cloning, and sequencing a region of 16S rDNA using primers designed to select against plastid and cyanobacterial sequences. Bacterial sequences were recovered from thirty-two of the forty host cells. For comparison, sequence libraries were also constructed for samples of the free-living and particle-associated bacterial assemblages. Network connectivity and sequence-based statistical analyses were conducted to assess similarities and differences among diatom host cells with regard to their bacterial associates, and among bacterial phylotypes with regard to their typical hosts. The data suggest host-cell specificity in one bacterial genus (*Arthrobacter*), which was found predominantly on *Thalassiosira* spp. cells, but most bacterial phylotypes were not specific to *Thalassiosira* spp. or other diatom hosts, and there was substantial variation in bacterial assemblages even among

closely related host cells. Principal coordinate analyses suggest that libraries derived from individual host cells can be placed in distinct groups that are explained by the phylogenetic relatedness of their component bacteria. That is, each group of libraries included a suite of closely related bacteria that were found in most libraries within the group, and were almost exclusively found in that group. Other phylotypes were found in more than one group and did not appear to be diagnostic of any one group. I propose that there is strong evidence for the existence of identifiable assemblages of bacterial phylotypes attached to diatom host cells; further work must be done to validate this hypothesis. As yet, the functional implications are unknown.

## **INTRODUCTION**

Algal-bacterial interactions have been studied for decades (Bell & Mitchell, 1972; Delong, Franks & Alldredge, 1993; Grossart et al., 2005), and the communities of bacteria closely associated with diatoms have been found to be distinct from free-living bacteria (Grossart 1999; Grossart et al., 2007). I argue that diatoms, their attached bacteria, and viruses associated with either host or bacteria may constitute a metaorganism as described by Bosch et al. (2011). Paraphrasing Bosch et al. and others (e.g. Biagi et al., 2011), a metaorganism is a polygenomic, composite organism derived from millennia of co-evolution with microbes. Host-microbiome symbioses are very well known in terrestrial systems (e.g. termites or ruminants and their cellulose-digesting gut microbes). In marine systems, the metaorganism concept has been studied extensively in coral and sponges where the term “holobiont” is used (Olson et al., 2010). Comparatively, very little is known regarding host-microbiome associations in diatoms (Grossart et al., 2010). If diatom-bacterial associations indeed act as a metaorganism, i.e. the properties of the diatom and bacteria acting together are distinct from each organism acting independently, then understanding this interaction may provide insight into the ecological and biogeochemical

roles of both diatoms and bacteria, including bloom formation (Smith et al., 1995) and the role that diatom-bacterial interactions have on the carbon cycle.

Presumably, environmental studies have always measured the net result of diatom-bacterial interactions, but their conclusions may be biased by a failure to recognize the true nature of that interaction. For example, the microbial loop follows dissolved organic matter (DOM) through a series of trophic pathways, with heterotrophic bacteria being the base of that pathway.

Phytoplankton set the rate of the microbial loop, as the rate DOM breaks down is constrained by the rate at which organic molecules are produced by phytoplankton. During preliminary work in this subject, Azam et al. (1983) assumed that bacteria remain at some distance from healthy diatoms (possibly because they produce antimicrobial agents) but attach to dead diatoms. We now know that diatoms and bacteria are often associated closely with one another through various life-stages (Grossart, 2010), with possible implications for the loci of bacterial metabolism (Grossart, 2010) and factors that maintain (Smith et al., 1995), influence or even cause diatom blooms. There is indirect evidence that diatom-bacterial interactions affected bloom duration and diatom biomass during a simulated bloom in a mesocosm, implying that diatom-bacterial interactions may also be important in the open ocean and affect ocean biogeochemistry (Smith et al., 1995).

Distinct groups of bacteria have adapted to living on surfaces in the open ocean (Blackburn et al., 1998; Delong et al., 1993; Mitchell et al., 1995; Grossart et al., 2007), and this association with surfaces must provide ecological advantages in exchange for the genetic load and metabolic cost of expressing genes associated with attachment. Even in the presence of adequate nutrients, some bacteria prefer surface colonization and invest in the production of antibacterial compounds to prevent competition with other species for the same surface (Yan et al., 2002). In the case of bacteria on diatoms, the association gives bacteria access to the

proteins and carbohydrates excreted by the diatom in addition to stability and safety (Rosowski, 1992). Bacterial morphology and metabolism change significantly to facilitate attachment to a surface, for example by producing large extracellular glycolipids (about 1 kDa) and glycoproteins (up to 100kDa) (Desai et al., 1997) that can be up to 10nm in diameter (Auerbach et al., 2000).

Previous studies have implied the possibility of bacterial-diatom associations forming a metaorganism (Bidle et al., 1999; Croft et al., 2005; Droop, 2007; Grossart et al., 1999; Rosowski 1992; Smith et al., 1995). These studies mostly focused on diatoms and bacteria acting in one of several possible modes of a classic symbiotic relationship, e.g. mutualism, commensalism, or parasitism, all of which are consistent with the metaorganism concept, and which may occur simultaneously in the same metaorganism. Vital nutrients, such as vitamin B<sub>12</sub>, are thought to be lacking in various marine environments; diatoms require this nutrient for growth and some are unable to produce it (Croft et al., 2005), therefore requiring an external source. Croft proposed that bacteria in the muciferous layer of *Thalassiosira pseudonana* provide the diatom vitamin B<sub>12</sub> and in return bacteria have a secure source of carbon, thereby forming a mutualistic relationship (Croft et al., 2005). More often a commensal relationship has been postulated, where diatoms are unaffected while bacteria have access to a secure carbon source (Droop, 2007; Rosowski 1992). Bacteria have been shown to produce enzymes that can cause dissolution of diatom frustules (Bidle et al., 1999), and some diatoms have been shown to have the capacity to produce antibiotics to ward off such bacterial parasites (Grossart et al., 1999).

Previously, marine diatom-bacterial interactions have been studied most often using cultured diatoms (e.g. Kogure et al., 1982; Grossart et al., 1999; Grossart et al., 2005; Kaczmarska et al. 2005; Grossart et al., 2007). Very few studies have been conducted using native populations of

diatoms (Kaczmarek et al., 2005). Furthermore, the concept of the diatom-bacterial metaorganism has yet to be explored in the oligotrophic open ocean, where nutrient limitation may lead to a greater importance of bacterial-diatom interactions, for example to the maintenance of a pertinacious species, the relative success of different diatom species, or the initiation and success of summertime blooms. My study site is within the North Pacific Subtropical Gyre (NPSG), which is considered to be the largest contiguous biome on Earth (Karl, 1999). To the best of my knowledge, no studies of bacterial-diatom associations have been conducted within a subtropical oligotrophic open ocean system. Furthermore, I am not aware of any application of single-cell approaches to examine the relationship of attached bacteria to diatom hosts.

## **METHODS**

### *Overview*

A concentrated sample of eukaryotic host cells was sent to an offsite facility that provides ultra-clean flow cytometric sorting and whole-genome amplification of DNA. Once host cells were identified, 16S rDNA from their associated bacteria was amplified, cloned, and sequenced. The resulting sequences were edited using Geneious® and identified using a SILVA alignment and ARB; these identities were used for a NodeXL network analysis. A phylogenetic tree of the 16S rRNA gene sequences was created by importing the ARB alignment into MEGA5, and served as the input data for analyses using the UniFrac software package. Detailed methods are provided below.

### *Study site*

The NPSG is an oligotrophic system with anticyclonic circulation from 15°N to 35°N and 135°E and 135°W. Samples were collected from Station ALOHA. The NPSG is a typical two-layer system; the bottom layer is nutrient rich, but light limited. In the well-lit surface layer, primary

productivity is supported by efficient nutrient recycling (Karl, 1999). Diatom populations in this system vary through the year in both species diversity and the abundance of individual species. Highest abundance of some species occurs in the summer months, especially in July (Scharek et al, 1999). Typical diatom species seen in blooms from June through September include *Rhizosolenia*, *Hemiaulus*, and *Mastogloia* (Dore et al., 2007). The causes of blooms in this system remain enigmatic and the methods for introducing the nutrients required to support an increase in biomass have yet to be revealed (Karl, 1999).

A feature of interest found in many systems, including the NPSG, is the deep chlorophyll maximum (DCM) (Cullen, 1982). The position of the DCM varies through the year, but is usually found around 100m. The DCM has been found to contain distinct diatom populations that have a high fucoxanthin to cell ratio. Diatoms in the DCM are primarily smaller pennate forms and appear less likely to sink out of the euphotic zone than the larger chain forming diatoms found in the mixed layer (Scharek et al, 1999). Whether or not the DCM is a stable environment is still contended. Although vertical mixing is reduced in the DCM, it is still an area that cells traverse as they fall from the mixed layer, as well as an area of high nutrient flux (Huisman et al, 2006). However, this flux may lead to higher diatom diversity (Huisman et al., 1999). As a recurring structure, the DCM was incorporated into my sample design, in part because it is an area of high chlorophyll per cell concentrations and typically smaller diatoms; both factors are particularly useful for flow cytometry, as described below. The dynamics of the system may also result in interesting diatom-bacterial interactions.

#### *Test of the cell concentration protocol*

The abundance of eukaryotic cells in the oligotrophic waters of Station ALOHA is relatively low, and requires an initial cell concentration step for effective flow cytometric sorting. The concentration step could result in loss of attached bacterial cells. To evaluate this possibility,

tests were conducted with two non-axenic cultures of diatoms (one pennate and one centric). The diatoms were collected on 25mm diameter, 5  $\mu\text{m}$  pore-size Nuclepore™ polycarbonate membrane filters (Whatman, Florham Park, New Jersey) and subsequently rinsed with 0.5 L of 0.2  $\mu\text{m}$  filter-sterilized seawater. The number of attached bacteria was assessed through 4',6-diamidino-2-phenylindole (DAPI)-staining. The associated bacteria on thirty different diatoms were counted for each of three treatments: unfiltered diatoms, diatoms collected on filters, and diatoms on filters that were subsequently rinsed with filtered water. Unfiltered diatoms were obtained by micropipetting, and were then DAPI-stained and examined using epifluorescence microscopy.

#### *Sample collection and concentration*

The final protocol used for field sampling was as follows. Samples were collected from Station ALOHA from July 8-10, 2010, during Hawaii Ocean Time-series (HOT) cruise 223 (*R/V Kilo Moana* cruise 1012). The DCM was sampled once per day for three days, and these samples were pooled in later analysis. On each day, 3 replicates of 3 L volumes were collected by gentle peristaltic pump filtration onto a 25 mm diameter, 5  $\mu\text{m}$  pore-size Nuclepore™ filter. While still moist, filters were rinsed with 0.5 L of filter-sterilized seawater. One of these filters was set aside as a sample of all particle-associated bacteria, including any bacteria on diatoms and other host cells, as well as bacteria associated with non-living particles. Smaller particles including bacteria passing through the 5  $\mu\text{m}$  filter were captured on a 25 mm diameter, 0.2  $\mu\text{m}$  pore-size Nuclepore™ filter, and represent the free-living bacterial assemblage. Filters were immediately placed in RNeasy lysis buffer (Qiagen, Valencia, CA), kept at room temperature overnight, and then stored at -20°C as recommended in the RNeasy protocol.



### *Cell sorting and whole genome amplification*

Host cells were gently re-suspended from the collection filter into RNAlater® prior to being sent to the Bigelow Laboratory Single Cell Genomics Center (SCGC) for cell sorting and subsequent genomic amplification. The SCGC is a specialized facility that operates a DNA-free clean room to minimize the possibility of contamination during the sorting operation and initial amplification.

At the SCGC facility, host cells were separated from the RNAlater® buffer by gravity filtration through a 10 µm mesh-size cell strainer (Becton Dickinson, Franklin Lakes, NJ, USA), and resuspended in UV treated seawater that was collected from Station Aloha and filter-sterilized by tangential flow filtration. Single host cells were sorted into wells containing 0.6 µL Tris-EDTA (TE) buffer by fluorescence-activated cell sorting (FACS) using a MoFlo (Beckman Coulter, Danvers, MA, USA) flow cytometer with a 488 nm argon laser for excitation, a 200 µm nozzle orifice and a CyClone robotic arm for droplet deposition into microplates. Because the Bigelow facility had not previously sorted cells within the size range expected for diatoms, preliminary testing was conducted to establish sorting parameters that would select in favor of diatoms from Station ALOHA. Final gating of cells for FACS was based on strong chlorophyll *a* signals indicative of active cells, and forward scatter indicating larger cell volumes (20-100 µm). Cell sorting was followed by DNA extraction using protocol A outlined by Stepanauskas and Sieracki (2007). Using heat stress, cells were lysed and DNA denatured in the course of three cycles of 97°C followed by 8°C. An 8-h multiple displacement amplification (MDA) was performed as described in the REPLI-g Mini kit (Qiagen, Chatsworth, CA). For each well, 5 µl of phosphate-buffered saline (PBS) containing the host cell and its bacterial associates had 0.4 µl of φ29 DNA polymerase, 14.5 µl of 1.7X reaction buffer, and 5µl of DNA-free deionized water added. The reaction mixture was incubated for 8 hours at 30°C, followed by deactivation of the polymerase at 65°C. The SCGC's MDA procedure includes a real-time screen of DNA production based on fluorescence of a DNA-specific stain. As a measure of MDA reaction kinetics, the SCGC reports

the value  $C_p$ , corresponding to the time required to reach the midpoint between background fluorescence and the maximum fluorescence signal. Wells with either rapid ( $C_p < 7$  min) or intermediate ( $7 < C_p < 12$ ) reaction kinetics are more likely to contain successfully amplified whole genomic DNA than wells with relatively slow reaction kinetics ( $C_p > 12$  min) (Ramunas Stepanauskas, SCGC Director, pers. comm.).

Following MDA, samples were verified to contain at least 100 ng DNA per  $\mu$ l before being diluted 1:100 in TE buffer and stored at  $-20^\circ\text{C}$ . Components in the REPLI-g Kit interfere with optical density (OD) measurements; DNA was quantified by fluorometry after staining with Quant-it™ PicoGreen® (Invitrogen, Grand Island, New York). The SCGC tested all wells with a real-time PCR screen using 18S rDNA primers Euk528F and Euk B (Medlin et al., 1988; Zhu et al., 2005) to identify wells that contain eukaryotic rDNA. Wells that were 18S-positive were Sanger-sequenced to identify the host cells, and the sequences were provided by the SCGC as part of their service.

### *Bulk environment samples*

As described earlier, particle-associated and free-living bacteria were obtained from the same source water as was used for single cell amplification. Both of these samples were extracted using a guanidinium-based lysis buffer and adsorption to a silica spin column (DNeasy Blood and Tissue Kit, Qiagen®), and were processed as described for MDA-amplified material, starting from the point of 16S rDNA cloning and sequencing.

### *Amplification and cloning of 16S rDNA*

The MDA-amplified DNA includes mitochondrial and chloroplast 16S rDNA associated with the host cell. Based on similarities between chloroplast and other bacteria-derived 16S rDNA sequences, I expected that PCR amplification of bacterial 16S rDNA might be overwhelmed by

the host cell's plastid 16S rDNA. When field-collected diatom samples were amplified using conventional 16S rDNA primers, 95% of the sequences were identified as chloroplast 16S rDNA (data not shown). Hodkinson and Lutzoni (2009) identified an 895F primer sequence to amplify bacterial 16S rDNA present in lichen (a fungal/algal symbiosis) without interference from chloroplast rDNA. The primer strongly discriminates against plastid and cyanobacterial 16S rDNA. The 895F primer was investigated using Primer Prospector (Walters et al., 2011) and select families were further investigated using ARB.

To increase the concentration of target DNA, a nested PCR protocol was developed using a first round of amplification with the 8F/1513R primer pair (Turner et al., 1999; Weisburg et al. 1991), followed by the 895F/1391R primer pair. The master mixes for both amplifications were similar to the recommendations outlined by the Platinum® Taq Polymerase users' manual (Invitrogen, Grand Island, New York), with the exception of increasing the concentration of MgCl<sub>2</sub> to 2.5 µM. The amplification for the first round was run according to the Platinum® Taq Polymerase users' manual, with 95°C for 3 minutes followed by thirty cycles of 94°C for one minute, 55°C for one minute, 72°C for one minute, and a final extension step of 72°C for seven minutes. The first-round PCR product was diluted in 1/500 in sterile water and re-amplified as described by Hodkinson and Lutzoni (2009) using the 895F/1391R primer pair. The protocol outlined by Hodkinson and Lutzoni starts with a less specific annealing temperature and then gradually increases specificity in each round. The PCR amplification was initiated by a 3 min denaturation step at 94°C, followed by 24 cycles that proceeded as follows: 94°C for 30 sec, 55°C for 30 sec (decreasing by 0.4°C with each cycle) and 1 minute at 72°C (increasing by 2 sec with each cycle). This was followed by 12 cycles of 94°C for 30 sec; 45°C for 30 sec; 72°C for 120 sec, increasing by 3 s with each cycle; and a final extension step of 10 min at 72°C.

The PCR product was separated on a 1.3% agarose gel in 0.5X TAE buffer and the product of the correct size was excised and purified using the PureLink™ PCR Purification Kit (Invitrogen, Grand Island, New York). The product DNA was cloned using a TOPO TA Cloning Kit® (Invitrogen, Grand Island, New York) and 30 unidirectional sequences per host cell and 50 per particle-associated or free-living library were obtained via Sanger sequencing on an ABI 3730XL at the Advanced Studies of Genomics, Proteomics and Bioinformatics Sequencing Services located at the University of Hawaii at Manoa.

#### *Evaluation of cell sorting and MDA*

The quality of the sorting process and the sterility of processing was tested upon return of the sorted, MDA-processed samples to the University of Hawaii. A set of wells in which no 18S rDNA had been amplified was tested for the presence of bacterial 16S rDNA. These included forty wells that were intended to receive a host cell, but had not resulted in successful recovery of 18S rDNA, and 15 wells that were intended as negative controls and were not expected to contain host cells. Samples from each were amplified using the PCR protocol described above. 16S rDNA was successfully amplified from some wells. Subsets of these 16S rDNA-positive wells were chosen for cloning and sequencing to determine the identity of the bacteria. Eight clones were selected and sequenced from each of six of the intended host-cell wells, and from each of seven of the intended negative-control wells.

#### *Data analysis*

18S rRNA gene sequences were evaluated using the NCBI database BLAST (Altschul et al., 1990) and identities were assigned based on the result with the highest sequence identity. A tree of 18S rRNA sequences was constructed using Geneious® (Drummond et al., 2012) Tree Builder at a 93% similarity using a global alignment, the Jukes-Cantor genetic distance model, and the Neighbor-Joining tree building method. 16S rDNA sequences were evaluated and

edited using Geneious® software, and were saved in a fasta file for later processing. Sequences were aligned using the Silva INcremental Aligner (SINA), which compares sequences to a quality checked reference tree (Silva Release 108 SSU Ref tree) and then compares sequences to 40 of its nearest neighbors before placing the sequence in the alignment (Pruesse et al., 2007). The SINA alignment was refined further in ARB based on agreement with the consensus and correct molecular folding. A small number of sequences could not be aligned and were assumed to represent amplification of non-target DNA or were chimeric and were removed from the analysis. Sequences identified as being of mitochondrial or chloroplast origin were also removed from further analysis. The remaining sequences were identified using ARB (Ludwig et al., 2004), with a filter limited to the positions amplified (26989 to 42549; *E. coli* SSU 16S DNA positions 880 to 1408). Sequences were grouped into phylotypes of 98% percent sequence identity (PSI) using FastGroupII, employing an algorithm that compares the similarities between two sequences and divides the matches found by the total number of bases in that sequence in a pair-wise fashion (Yu et al., 2006). The identified phylotypes were used to conduct a network analysis using NodeXL (Smith et al., 2009), a visualization tool to examine how communities are interconnected.

Additional analyses were performed independently of taxonomic assignments and were based on sequence relatedness. Some sequences did not include both primers and were removed prior to statistical analysis. The remaining sequences were exported from ARB as an aligned fasta file with gaps, using the ECOLI filter with the positions restricted to 26989 to 42549, which includes 528 base positions. A phylogenetic tree was then built from the alignment using MEGA5 (Tamura et al., 2005), which has the advantage of computational speed. The tree that best fit the ARB identification was a maximum likelihood tree calculated using the Jukes-Cantor base substitution model, assuming a uniform evolution rate at all sites. Positions were deleted

when fewer than 95% of sequences had a base at that position (i.e. more than 5% had missing data).

The phylogenetic tree produced by MEGA was used as input data for analyses using the UniFrac package (Lozupone et al, 2006), which provides a set of tools to compare microbial communities based on phylogenetic information. I employed the UniFrac P-test for all libraries followed by pairwise P-tests between libraries, Principal Coordinate Analysis (PCA), and environment clustering. The goal was to assess the statistical similarities and differences among bacterial sequence libraries derived from eukaryotic host cells or representing free and particle-associated bacteria. P-tests are used to evaluate whether environments are different from one another, using Monte Carlo methods to remove sequence dissimilarities and calculate significance. PCA is used to assess causal relationships by placing samples in orthogonal, multidimensional space, where each dimension identifies variability in the data in order of most important to least. Environmental clustering is used to assess and rank environments (in this case, individual host cells) in order of the phylogenetic relatedness of their microbial communities.

#### *Repeat sequences*

In many analytical approaches used to compare libraries derived from different samples, the composition of each library can be weighted by the number of times a particular sequence occurs. Due to the inclusion of MDA in the methodology, the number of times a given sequence was found in a clone library is not expected to have any relationship to the number of times the corresponding bacterium appeared on its host cell. Sequence abundance is therefore ignored in my community level analyses. All analyses described herein are based solely on the presence or absence of phylotypes in libraries.

## RESULTS

### *Effect of filtration on the numbers of attached bacteria*

The numbers of bacteria remaining attached through the filtration process were assessed using DAPI stained cultures of both pennate (from Station ALOHA—test 1) and centric (from Kaneohe Bay, Oahu HI—test 2) diatoms. For test 1, twenty-five diatoms collected directly from culture (i.e. there was no filtration step) were compared to thirty diatoms that had undergone filtration followed by washing with 500 mL of 0.2  $\mu\text{m}$ -filtered seawater. Diatoms lost 55% of their associated bacteria during filtering and washing (t-test,  $p < 0.01$ ) (Figure 1A). Test 2 (Figure 1B) examined the effect of washing the filtered diatoms to remove non-attached bacteria from the filter. Although there was a small reduction in the mean number of bacteria per host cell at the highest wash volume tested (400 mL), compared to bacteria present immediately after the filtration process, the loss was not statistically significant ( $F = 1.41$ ,  $p = 0.24$ ).

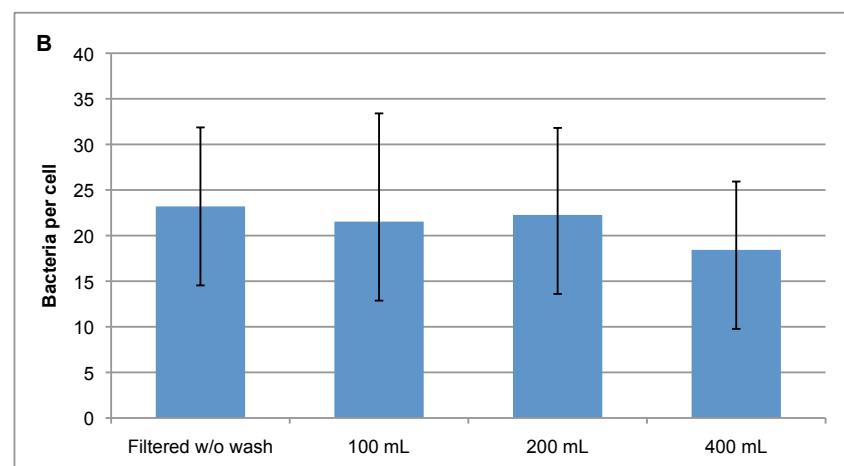
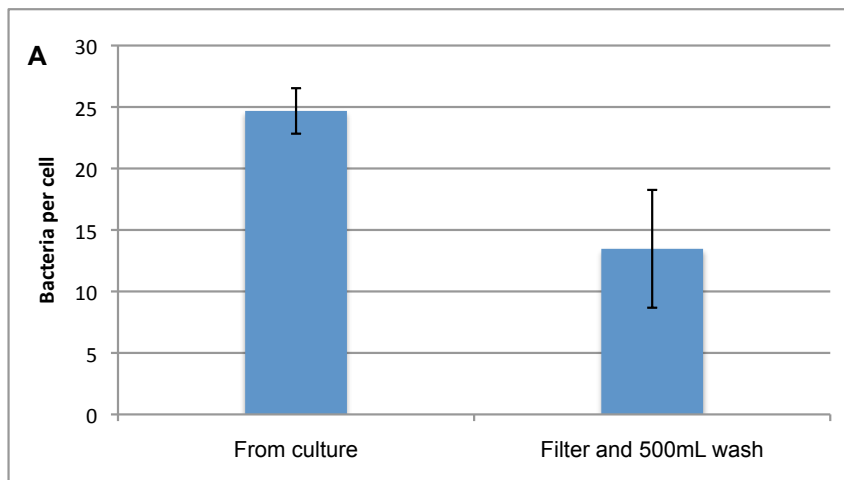


Figure 1. The effect of filtering and rinsing on the bacteria associated with diatom cells. (A) Test 1: Compares cells taken directly from their environment (culture) to cells collected on a filter, then washed. (B) Test 2: Compares the effect of additional washing steps following initial capture on a filter.

Because the effect of the washing on cells was not statistically significant, the four different treatments were pooled to provide a frequency distribution of the number of bacterial cells per host cell (Figure 2).

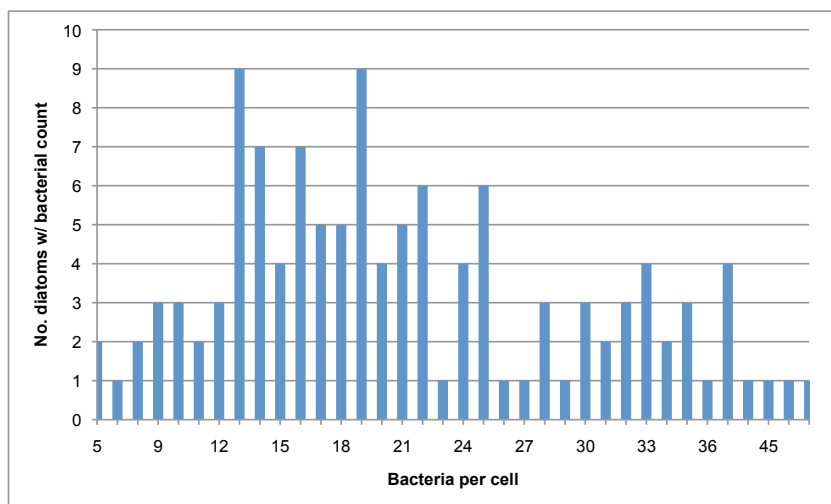


Figure 2. Frequency distribution of the number of bacterial cells per host diatom cell. Same individual cells as in Figure 1, Test 2.

Following collection on a filter, from 5 to 46 bacterial cells were attached per diatom (mean = 24). Visual inspection of cells before and after filtration indicates that the lost cells were probably loosely associated. The bacteria that remained after the initial filtration remain attached even after repeated rinsing. Washing was effective at removing unattached bacteria: few or no bacteria were observed on the filters following washing.

#### *FACS and MDA results*

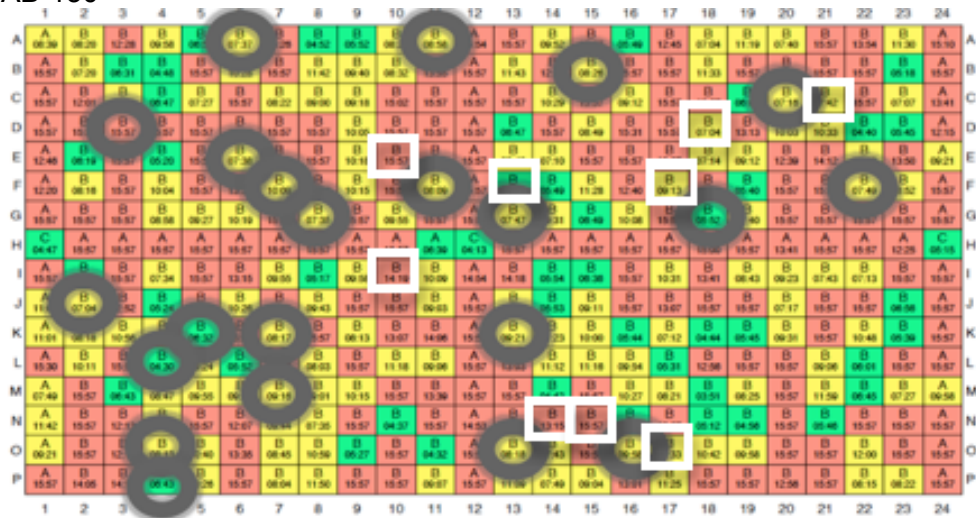
Two plates of 384 samples were produced by the FACS and MDA, and are color-coded in Figure 3 to mark rapid (green), intermediate (yellow) and slow (red) MDA kinetics. Slow MDA reactions are likely to have failed, either by a failure of the sorting process or a failure of the subsequent amplification. MDA reactions that are marked as either yellow or green are equally likely to produce enough DNA for downstream analyses (Ramunas Stepanauksas, pers.



comm.). Columns 1 (left edge), 12 (middle), and 24 (right edge) and row H (center row) are negative controls and no cells were intentionally sorted into those wells; well H12 is a positive control that is intended to receive 10 cells during sorting. Both plates underwent 18S rDNA real-time PCR screening, and wells found to be 18S rDNA-positive were cloned and sequenced to ascertain the identity of the host cell. 18S rDNA was successfully recovered and identified from a total of 45 wells. Not all samples that had rapid or intermediate MDA kinetics proved to have an 18S rDNA positive signal using real time PCR, nor did those that had a positive real time PCR response necessarily produce valid 18S rDNA sequence (Table 1). From the 45 wells that yielded valid 18S rDNA sequences, all diatom and a subset of other host cells were chosen for further analysis (a total of 40 total host cells).

Figure 3. MDA results. Green cells were likely positives with a  $C_p$  value lower than 7, yellow cells were intermediate reactions with a  $C_p$  between 7 and 12, and red cells were likely to have failed with a  $C_p > 12$ . Wells circled in gray were successfully sequenced for 18S rDNA. Wells marked with a white box appeared to have a successful real time PCR amplification of 18S rDNA, but were not successfully sequenced.

AB-130



AB-132

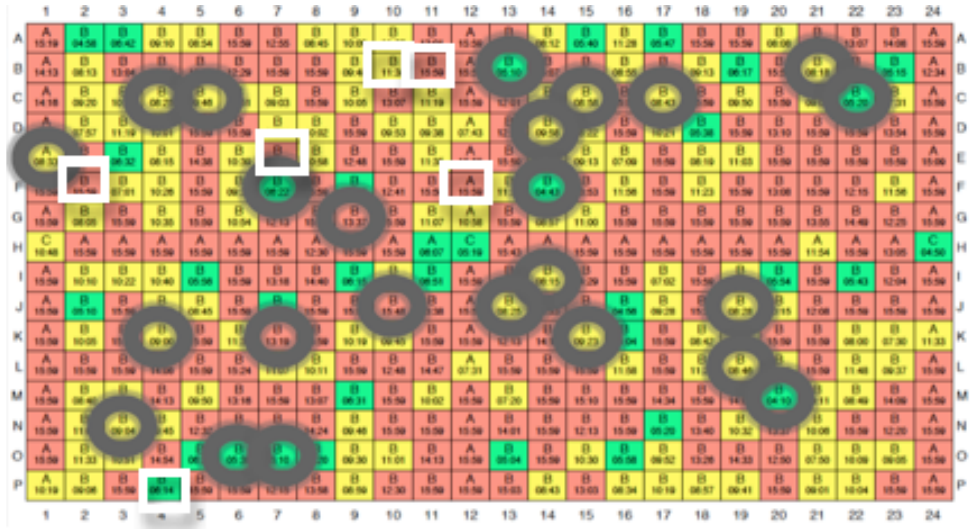


Table 1. A comparison of MDA kinetics (= MDA screen) and real time PCR screening for successful 18S rDNA amplification (= real time PCR+) compared to successful or unsuccessful sequencing of 18S rDNA (=18S sequencing + or -). The number of wells in each category are indicated. A total of 45 wells yielded a valid 18S rDNA sequence.

Plate name	AB-130	AB-132	Total
MDA screen (green)	48	35	83
Real time PCR +	5	8	13
18S rDNA sequence +	4	7	11
18S rDNA sequence -	1	1	2
MDA screen (yellow)	137	130	267
Real time PCR +	21	14	35
18S rDNA sequence +	17	13	30
18S rDNA sequence -	4	1	5
MDA screen (red)	199	219	418
Real time PCR +	5	7	12
18S rDNA sequence +	1	3	4
18S rDNA sequence -	4	4	8

Twenty-one of the forty cells that were intended to receive a FACS-sorted host cell were 16S rDNA positive, and seven of the negative control wells were also 16S rDNA-positive. Six of the former and all seven of the latter were cloned and sequenced (Table 2). Of those clones taken from wells that were intended to receive host cells, a chloroplast 16S rDNA sequence was found in one, and probable bacterial contaminants (*Propionibacterium*, *Haemophilus*, *Streptococcus*) in others. The remaining sequences appear to be possible marine bacteria including *Delftia*, *Chloroflexi*, *Simplicispira*, and *Skermanella* (although the last one has also been reported as airborne contaminants) (Weon et al., 2007). The negative-control wells primarily contained probable contaminants (e.g. *Staphylococcus*, *Propionibacterium*, *Streptococcus*, *Ralstonia*) but two of the wells contained possible marine bacteria (*Pseudomonadaceae* and *Massilia*).

Table 2. Recovery of bacterial 16S rDNA sequences from sample wells that were not intended to receive FACS cells (Negative), and wells that were intended to receive host cells (Positive) but did not have a retrievable 18S signal. A majority of the bacterial sequences retrieved from the Negative and “empty” Positive wells were probable contaminants.

Negative:

Sample #	ARB IDs found
1	<i>Staphylococcus</i> , <i>Propionibacteriales</i>
2	<i>Pseudomonadaceae</i>
3	<i>Staphylococcus</i>
4	<i>Massilia</i> , <i>Staphylococcus</i>
5	<i>Polynucleobacter</i> , <i>Ralstonia</i>
6	<i>Propionibacterium</i> , <i>Staphylococcus</i> , <i>Streptococcus</i>
7	<i>Skermanella</i>

Positive:

Sample #	ARB IDs found
1	<i>Acidovorax</i> , <i>Microsporidiomycota</i> (fungus), chloroplast
2	<i>Haemophilus</i> , <i>Chloroflexi</i>
3	<i>Delftia</i> , <i>Simplicispira</i>
4	<i>Skermanella</i> , <i>Streptococcus</i>
5	<i>Skermanella</i>
6	<i>Delftia</i> , <i>Propionibacterium</i>

### 18S DNA Sequences

Of the 40 host cells isolated and amplified using FACS and MDA, 33 were diatoms identified as 27 *Thalassiosira* spp., 3 *Chaetoceros* spp., and one each of *Pseudo-nitzschia* sp., *Guinardia* sp., *Leptocylindrus* sp., and *Delphineis* sp. The remaining host cells included dinoflagellates (*Dinophyceae*, *Prorocentrum triestinum*), coccolithophorids (*Calcidiscus leptoporus*), and flagellates (*Bicosoeca vacillans*, *Isochrysis*, *Solenicola setigera*) (Figure 4).

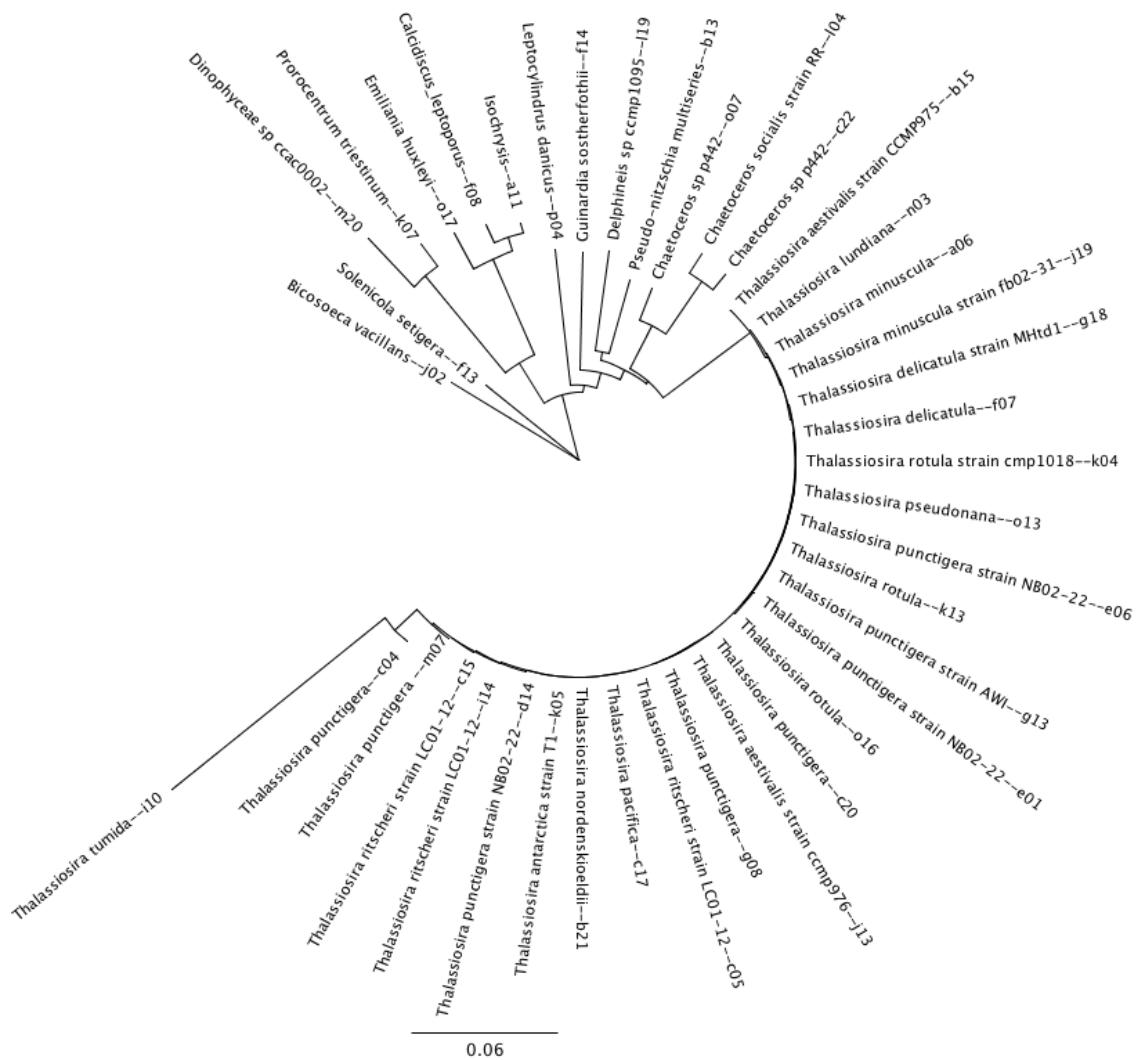


Figure 4. Host cells investigated in this study using the 18s rRNA gene. *Bicosoeca vacillans*—J2 was chosen as the root. The scale bar represents the number of amino acid substitutions per site.

### Investigation of 895F primer

The 895F primer was selected for use in this study because of its preferential amplification of non-chloroplast sequences. As noted by Hodkinson and Lutzoni (2009), when 895F is compared to sequences in the RDP-II Probe Match analysis, there is a 66.68% sequence coverage for a strict consensus and 91.07% coverage if a single mismatch was allowed. Using Primer Prospector (Walters et al., 2011) to investigate the primer match to the SILVA database, the 895F primer was found to have a strict consensus with 61.11% of the bacterial sequences, 63.54% of the archaeal sequences, and 0.01% of the eukaryal sequence (Figure 5).

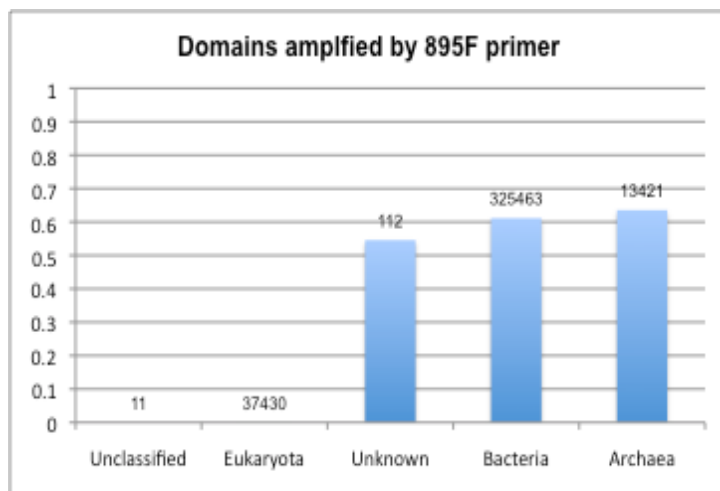


Figure 5. Predicted proportion of the domains of life that would be amplified by the 895F primer, based on Primer Prospector and the Silva database using a strict consensus. Values at tops of columns are the number of sequences in each domain.

In all sequences that included the 895F primer, nearly all variability occurred at the degenerate positions (5'-**CR**CCTGGGGAG**T**R**CR**G-3'). Most bacterial phyla that were found in previous work with diatom hosts would be amplified using the 895F primer under stringent conditions allowing zero mismatches to one of the sequences included in the degenerate 895F primer (Figure 6A). Most of the classes that have been identified in previous work would also be recovered, with the exception of *Sphingobacteria* and *Flavobacteria* (Figure 6B), which might be underrepresented.

I examined the primer match to families seen in previous studies of diatom-associated bacteria (*Pseudoalteromonas*, *Alteromonas*, *Flexibacteriaceae*, *Hyphomonas*, *Campylobacter* and *Roseobacter*), not all of were represented in the present dataset. The 895F primer will amplify most families previously documented with a greater than 98% coverage using a strict consensus, with the exception of the *Flexibacteriaceae* group, for which only 2.2% of the sequences are covered (Figure 6C). Although the 895F primer does not cover one group that was found in previous studies of bacteria associated with diatoms, it does cover the majority of previously documented groups. The practical advantage of discriminating against chloroplast 16S rDNA outweighs the disadvantage of reduced coverage of one group.

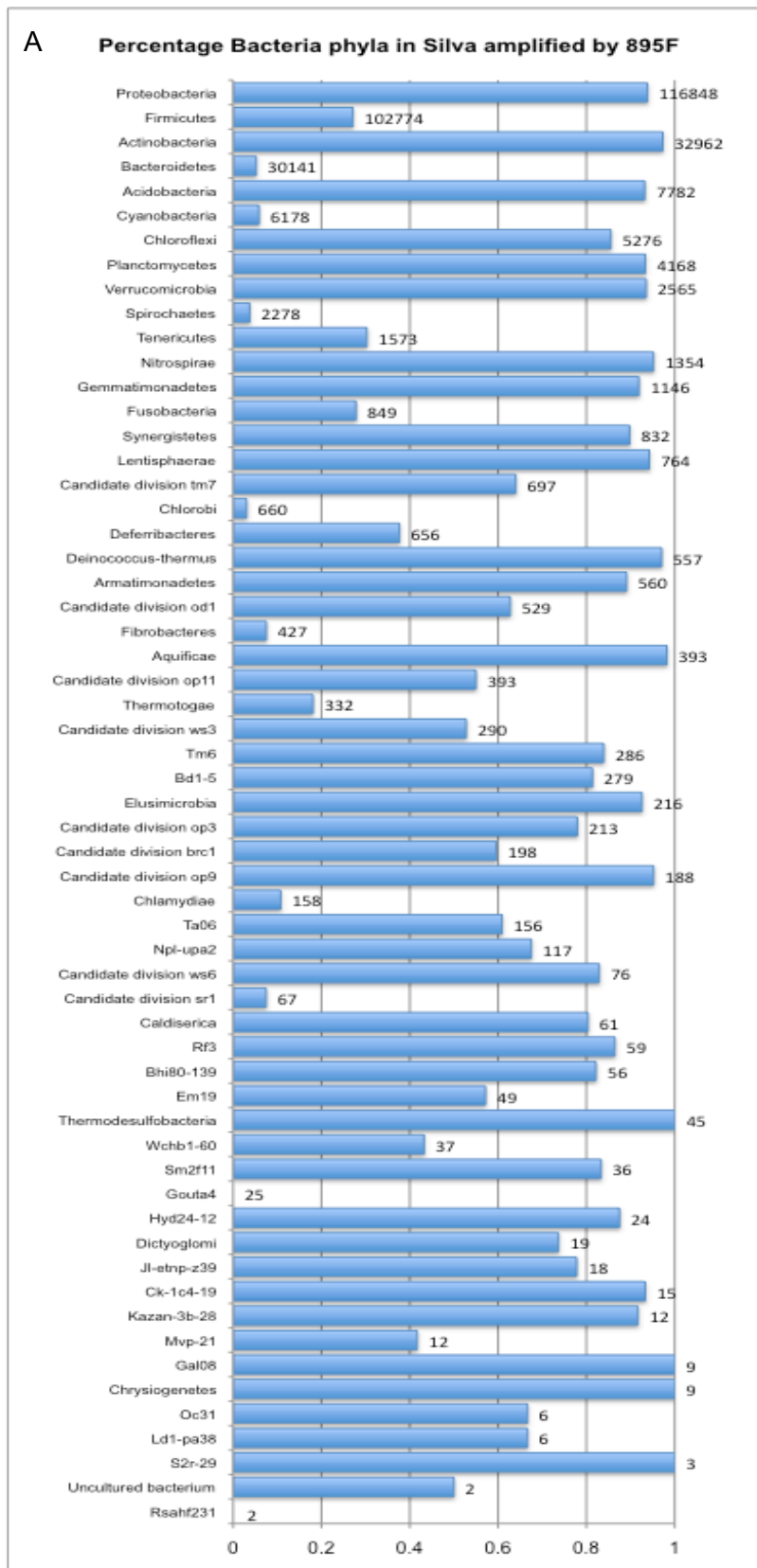
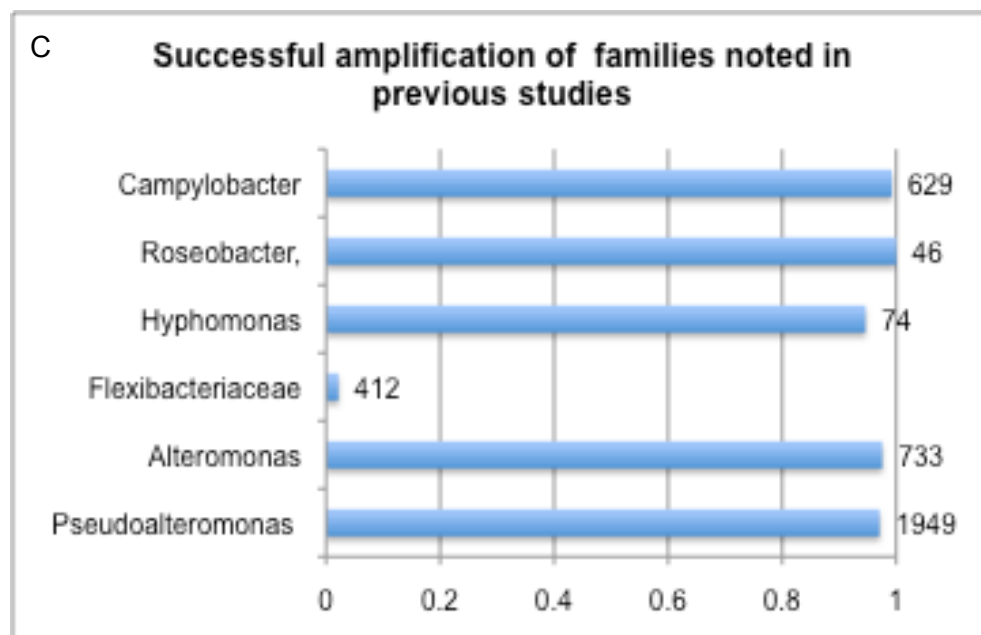
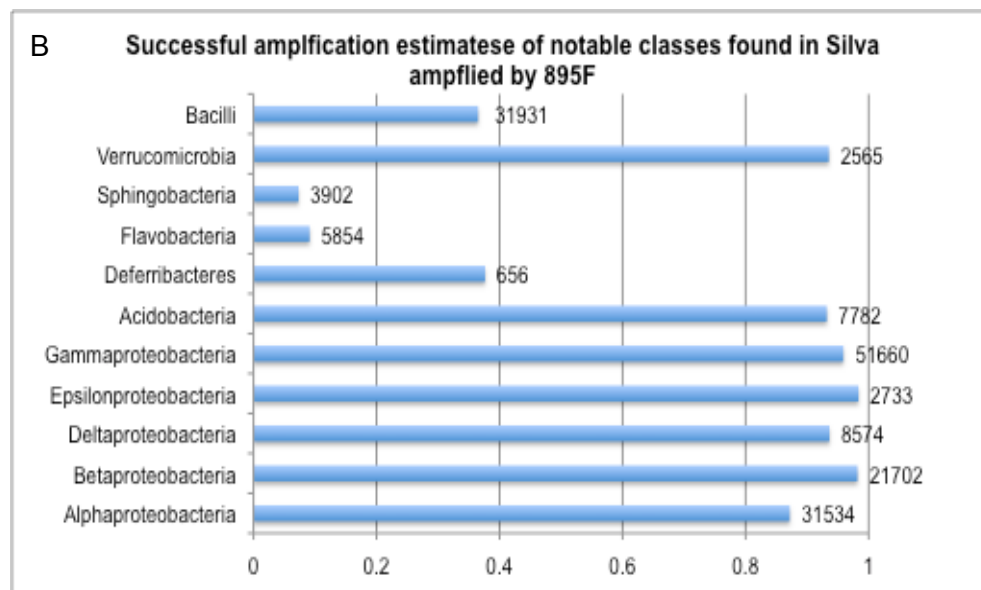


Figure 6. The results of the primer prospector output for 895F when compared to the SILVA database using a strict consensus, for A) phyla, B) classes, and C) families that were obtained in previous studies of diatom-bacteria interactions. The number of individuals in each group are labeled to the right of the bar graph.



### *Identity of 16S sequences*

A total of 1329 16S rDNA sequences were recovered from 40 host cells, one sample of particle-associated bacteria, and one sample of free-living bacteria (42 libraries in total). Sequences removed prior to statistical analysis included: 89 chloroplast, 506 mitochondria, 75 eukarya, and 47 possible contaminants (*Propionibacterium*, *Faecalibacterium*, *Clostridium*, *Lactobacillus*,



*Lactococcus*, *Streptococcus*, and *Synergistaceae*). Additional sequences were removed because the amplified fragment was out of the 26989 to 42549 positions (i.e. an alternative 16S target site existed for this primer pair) or because sequences could not be aligned with the ARB database (i.e. probable amplification of non-target, non-16S DNA). Within the remaining 424 sequences, 196 different phylotypes were identified at 98% percent sequence identity (PSI). These 196 different phylotypes were identified by ARB as having 68 unique taxonomic identities, typically matching existing sequences at the genus level. The 424 sequences used for statistical analysis are listed in the Appendix (Table I), with their ARB-based identification and the nearest environmentally relevant sequence.

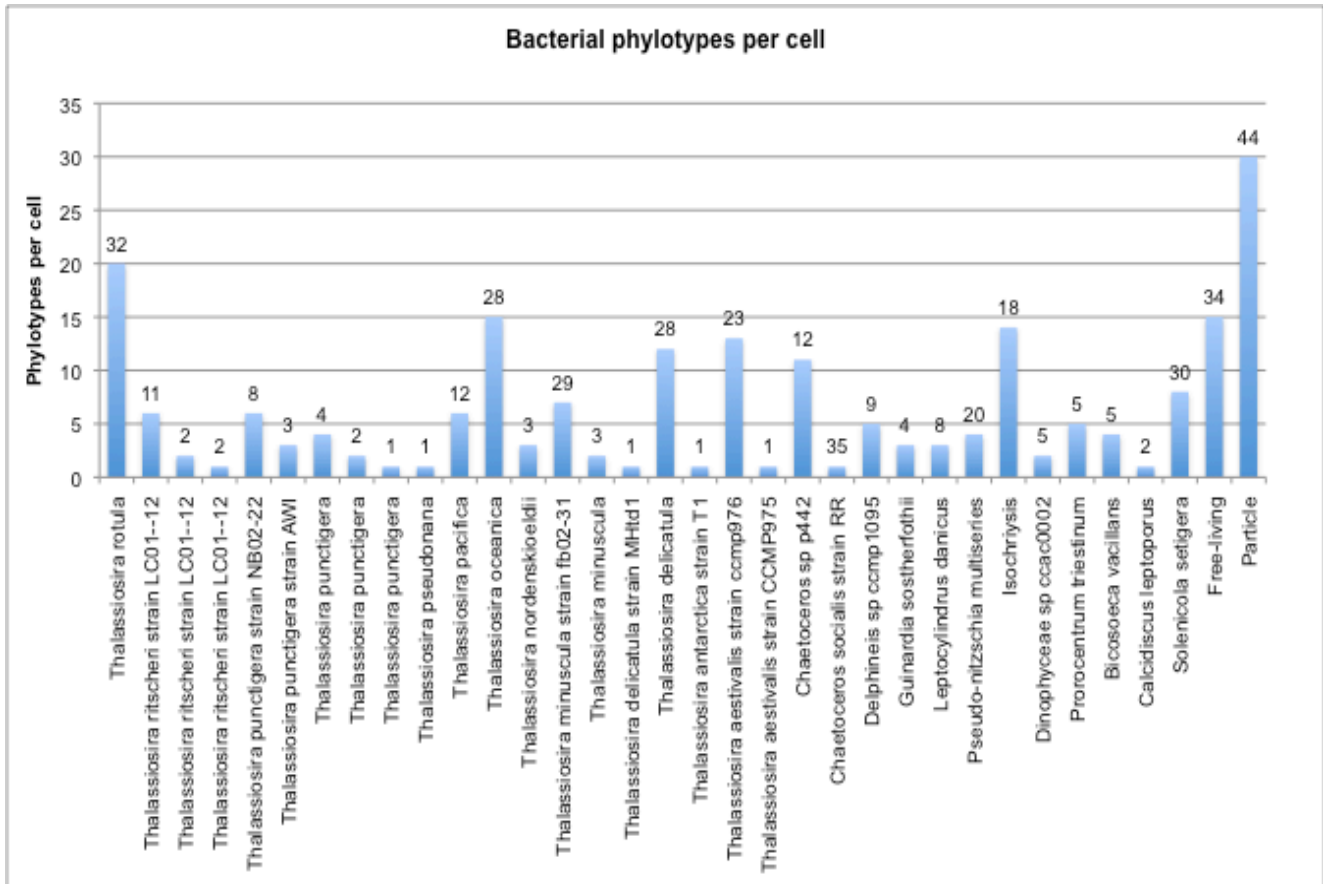


Figure 7. Bacterial phylotypes per library. From left to right: *Thalassiosira* host cell libraries; other diatom host cell libraries; non-diatom host cell libraries; free-living bacterial library; particle-associated bacterial library. The number of valid sequences found per cell is provided at the top of each column.

No bacterial sequences were recovered from eight of the host cells (7 *Thalassiosira* and 1 *Chaetoceros*). The 32 remaining host-cell libraries contained from 1 to 20 different bacterial phylotypes, with the greatest number of bacterial phylotypes attached to a *Thalassiosira rotula* host cell (Figure 7). The particle-associated library contained 30 different phylotypes, and 15 different phylotypes were present in the free-living library.

#### *Phylotypes shared in different host cell libraries*

Network analysis provides a useful visualization of the degree to which the bacterial assemblages on cells are connected (Figure 8), and is shown for the 34 different libraries. Six *Thalassiosira*-derived libraries did not have any bacterial phylotypes in common with another cell (Figure 8). All other cells had at least one shared phylotype. Two *Arthrobacter* phylotypes were found associated with several host cells. Only one bacterial phylotype was recovered from eight of the 32 host cells with associated bacteria; these included 6 *Thalassiosira* spp., 1 *Chaetoceros* sp., and 1 *Calcidiscus leptoporus* host cell. Only one phylotype was found in both the free-living and particle-associated libraries, and only three phylotypes were found in a host cell library and either the free-living or particle-associated library.

At either the phylotype (Table 3) or class level (Table 4) I found little evidence of specific associations between a bacterial group and a host cell type; most bacterial groups occurred on more than one host cell type. *Arthrobacter* is the one notable exception, as it appears on 1/3 of the *Thalassiosira* cells and on only one other host cell.

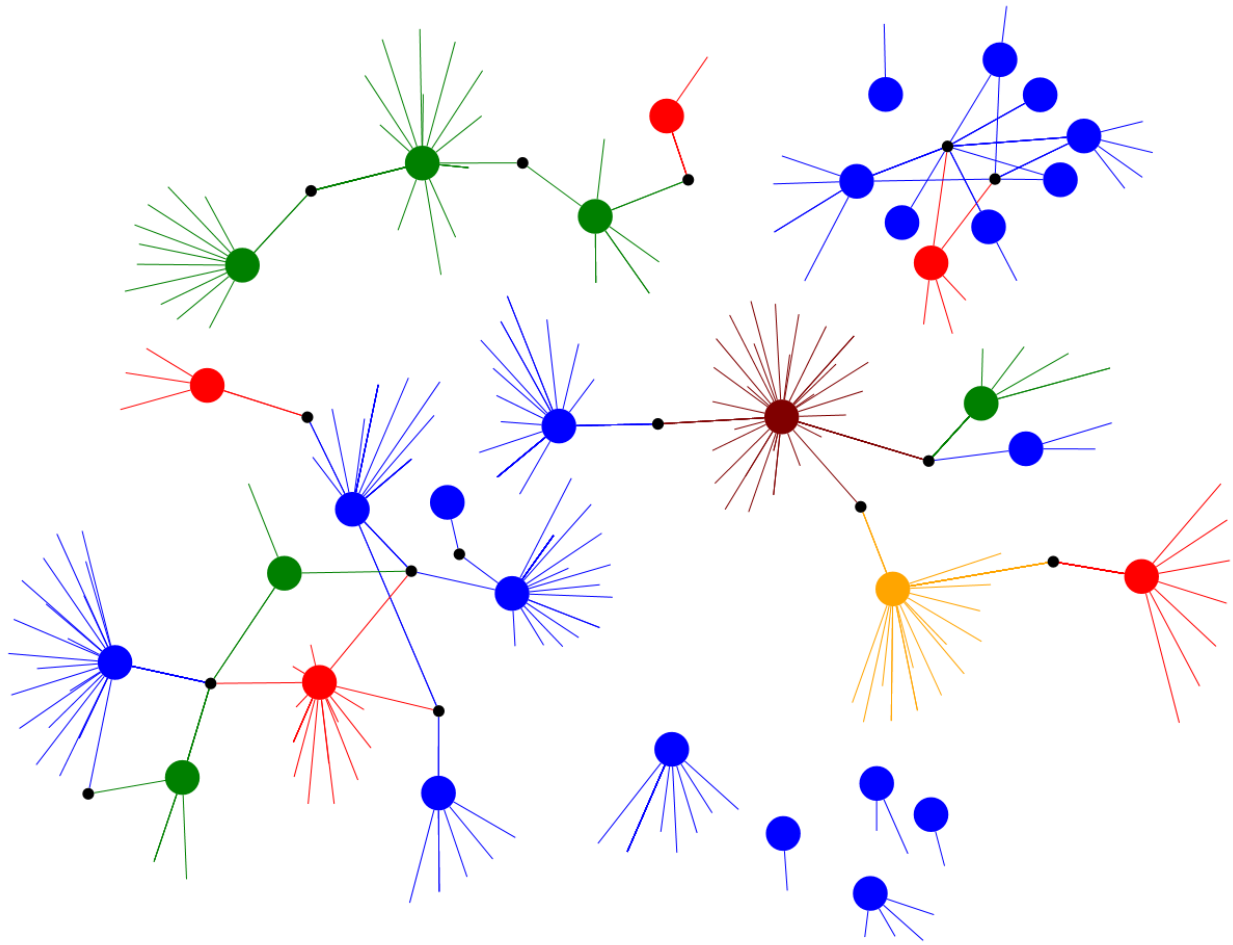


Figure 8. Network visualization. Libraries are presented by color-coded nodes and associated bacterial phylotypes by radiating lines. Lines that touch at their respective ends indicate phylotypes found in two or more libraries. Libraries with no connections have no bacterial phylotypes shared with another library. The distance between libraries and the length of lines have no meaning. Blue = *Thalassiosira*; green = other diatoms; red = non-diatom host cells; black = shared bacterial phylotypes.

Table 3. Bacterial phylotypes as they are seen on different host cell types. Different phylotypes may have identical names assigned by ARB, and are therefore separated in this study by the notation of type 1 and type 2.

Class	Last Arb-ID	Thalassiosira	Other diatoms	Other host cells	Particle	Free	Total
Actinobacteria	<i>Arthrobacter</i> , type 1	7	0	1	0	0	8
Actinobacteria	<i>Arthrobacter</i> , type 2	4	0	1	0	0	5
Alphaproteobacteria	<i>Caulobacteraceae</i>	1	2	1	0	0	4
Betaproteobacteria	<i>Delftia</i>	2	1	1	0	0	4
Gammaproteobacteria	<i>Vibrionaceae</i>	1	1	0	1	0	3
Actinobacteria	<i>Brachybacterium</i>	2	0	1	0	0	3
Flavobacteria	<i>Cryomorphaceae</i> , NS7 marine group	0	0	0	1	1	2
Gammaproteobacteria	SAR86 clade	0	0	1	0	1	2
Betaproteobacteria	<i>Massilia</i>	0	1	1	0	0	2
Flavobacteria	<i>Tenacibaculum</i> , type 1	0	2	0	0	0	2
Flavobacteria	<i>Tenacibaculum</i> , type 2	0	2	0	0	0	2
Gammaproteobacteria	<i>Acinetobacter</i>	1	0	1	0	0	2
Gammaproteobacteria	<i>Pseudomonas</i>	1	0	0	1	0	2
Alphaproteobacteria	<i>Caulobacteraceae</i>	1	1	0	0	0	2
Sphingobacteria	<i>Sphingobacterium</i>	2	0	0	0	0	2

Table 4: Bacteria at the level of class as they are seen on different host cell types.

Bacteria (Class ID)	Thalassiosira	Other diatom cell	Other host cell	Free-living	Particle	Total
Actinobacteria	12	0	3	1	0	16
Gammaproteobacteria	6	1	2	1	1	11
Alphaproteobacteria	5	3	2	1	1	12
Betaproteobacteria	4	2	2	0	0	8
Sphingobacteria	2	0	0	0	0	2
Deltaproteobacteria	1	1	2	1	1	6
Bacteroidetes	1	0	0	0	1	2
Flavobacteria	0	3	0	1	1	5
Bacilli	0	1	0	0	0	1
Acidobacteria	0	0	0	1	0	1
Bacteroidia	0	0	2	0	0	2
Lentisphaeria	0	0	0	0	1	1
Planctomycetacia	0	0	0	0	1	1
Verrucomicrobia	0	0	0	0	1	1

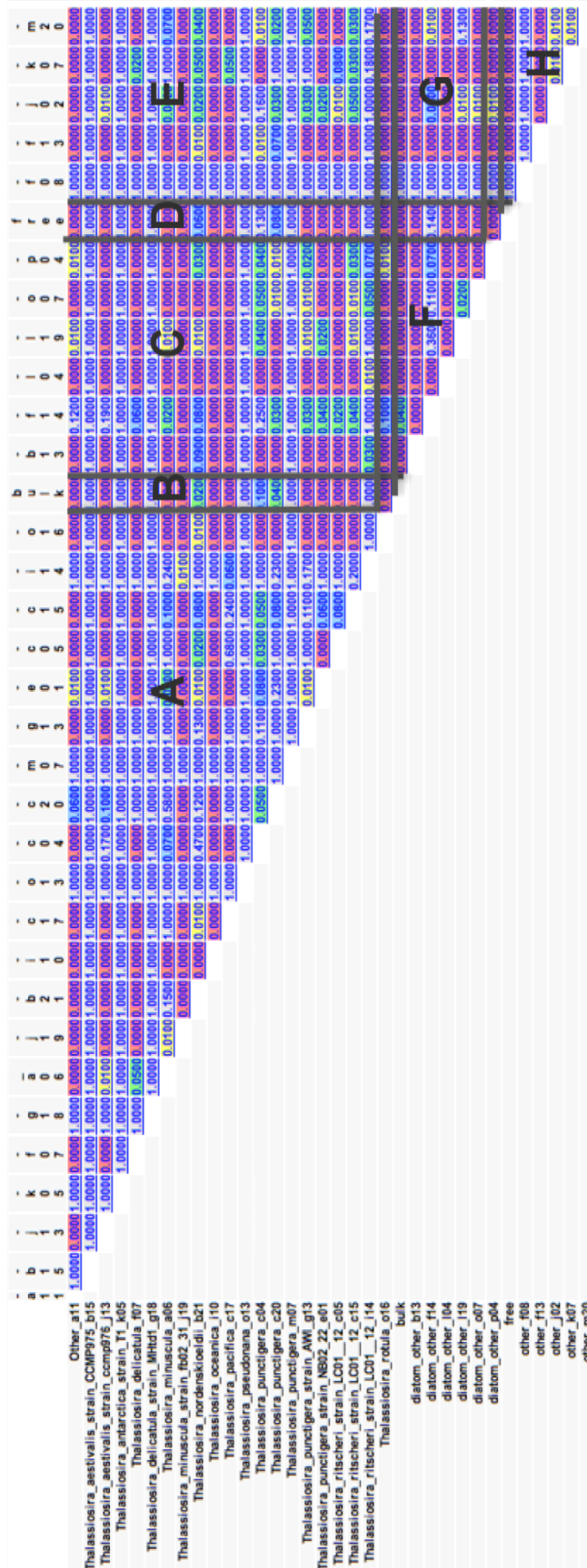
### *Microbial community comparison using UniFrac*

The analyses described above provide insight into the makeup of bacteria associated with host cells, but do not test whether significant variability occurs among these bacterial assemblages. I employed P-tests, PCA, and environmental clustering for a more robust assessment of similarities and differences among libraries.

Significant variability occurred among the 34 libraries as a whole ( $P < 0.001$ ; P-test among all libraries as described by Martin 2002). Of the 528 possible pairwise comparisons among libraries, a majority were not significantly different, especially between libraries derived from *Thalassiosira* host cells (Figure 10; pairwise P-tests; blue color denotes non-significant pairs); however, some *Thalassiosira*-derived libraries differed significantly from one another. The rest of the diatom libraries were generally different from non-diatom host libraries. From this first look, I can infer that libraries derived from diatom host cells tend to be similar to one another, but there is considerable variability even among closely related host cells.

Pairwise comparisons do not tell the full story, as libraries that are significantly different can still fall into groups that are relatively 'less different' within the group. Principal Coordinate Analysis based on bacterial 16S rDNA sequences suggests that host cell libraries fall into identifiable groups (Figure 11). PCA axes 1 through 3 together explain 40% of all variability in library composition.

Figure 10. Pairwise P-tests among all libraries; P-values are corrected for multiple comparisons using the Bonferroni correction. Red, yellow and green cells denote pairs of libraries that differed significantly at  $p < 0.05$ . ; A- *Thalassiosira* vs *Thalassiosira*; B- *Thalassiosira* vs. particle-associated bacteria; C- other diatoms vs *Thalassiosira* D- *Thalassiosira* vs. free-living bacteria; E- *Thalassiosira* vs non-diatoms; F- Other diatoms vs. other diatoms; G- other diatoms vs non-diatom libraries; H- non-diatom libraries vs. non-diatom libraries.



PCA often proceeds by identifying environmental control variables that are highly correlated with the first several PCA axes and are likely causes of the observed variations. Because all libraries were drawn from the same water sample, the only information available to characterize these PCA groups is the composition of libraries within each group. Factors that might influence library composition include host cell identity, the diversity of bacterial phylotypes within libraries, the distribution of unique bacterial phylotypes among libraries, the distribution of phylotypes found in common with other libraries, and the phylogenetic relatedness of non-identical bacteria in libraries.

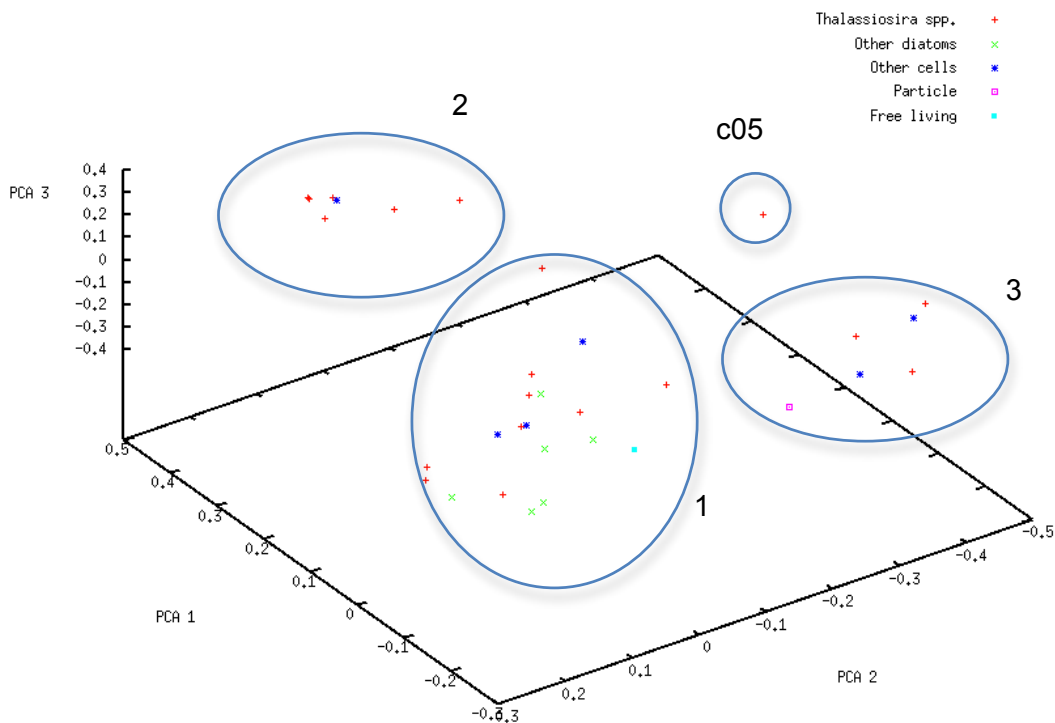


Figure 11. Principal Coordinate Analysis. 40% of variation is explained by the first three axes, which divide the 34 libraries into three groups. Of the three axes, 20% of variation is explained by PCA axis 1, 11% by PCA axis 2, and 9% by PCA axis 3. Host cell c05 (*Thalassiosira ritscheri* strain LC01—12) does not fall into any of the three groups. The PCA results imply both within-group similarities and between-group differences in library composition.

All PCA groups contain *Thalassiosira*-host libraries, and PCA group one includes all of the non-*Thalassiosira* diatoms. Non-diatom host cells appear in all three PCA groups. The particle-associated library and the free-living library cluster in different PCA groups, although they are close to each other in 3D space. It is clear that libraries are not based on host cell identity, so there must be an alternative reason for the groupings. The distributions of unique and shared phylotypes are consistent with what would be expected for the number of cells present in each PCA group (Table 4). The number of connections between cells is much lower for group 2 than would be expected based on the size of the group, and is somewhat higher than expected for group 1. Although these properties may contribute to the PCA groups, no defining characteristic is evident.

Table 4. Characteristics of 4 groups identified by PCA. **Total phylotypes in group** is the number of different phylotypes in each library, summed over all libraries within a group; each phylotype is counted only once per library. **Unique phylotypes** occurred only once in the entire data set. **Shared within group** denotes the number of phylotypes that appeared in more than one library within the group. **Connections within group** denotes the total number of times any bacterial phylotype was shared within a group, and is larger than the “shared within group” value because some phylotypes are shared more than once. **Expected\*** values were calculated based on the assumption that all phylotypes are randomly distributed among all libraries regardless of group.

\*I chose not to evaluate the statistical probability of the observed difference between expected and actual values, e.g. by Monte Carlo simulation, because I lack an appropriate independent data set to estimate probability distributions.

Group	Phylotypes				Shared phylotypes			
	Libraries in group	Total phylotypes in group	Unique	Expected unique	Shared within group	Expected shared	Connections within group	Expected connections
1	19	106	100	113	6	6	37	25
2	8	21	19	48	2	2	2	11
3	6	80	78	36	2	2	6	8

I investigated the association of bacterial classes with specific PCA groups using a subtractive PCA approach, in which the most abundant classes were removed one at a time from the PCA to determine whether any of them strongly influenced PCA grouping. Only classes that were found in numerous libraries are likely to affect entire groups. PCA grouping was strongly affected by removal of some classes from the analysis (Table 5). The greatest degree of group dissolution was found when removing *Actinobacteria* and *Flavobacteria* from the analysis (see



Appendix, Figure II-A and II-E). *Actinobacteria* were only found in 16 of the 34 libraries, and 26 different phlotypes were found, yet removal of those sequences resulted in the dissolution of most of the PCA groupings. *Flavobacteria* were found in 5 of the 34 libraries and 29 different phlotypes were found, but removal of these sequences again caused dissolution of PCA groups.

Table 5. Classes of prokaryotes found in each PCA group, plus one *Thalassiosira ritscheri* strain LC01—12 (c5), which did not cluster with any group. The values for each class are the number of libraries in which that class appeared in each group of libraries. The number of libraries per group is shown in parentheses. The classes that appeared in more than three groups (highlighted) were tested for the affect of their being removed from the PCA. The groupings that were affected are noted.

Class	Group 1 (19)	Group 2 (8)	Group 3 (6)	c05	Subtractive PCA
Acidobacteria	1	0	0	0	
Actinobacteria	4	8	3	1	1 & 2
Alphaproteobacteria	8	0	4	0	2 & 3
Bacilli	1	0	0	0	
Bacteroidetes	1	0	1	0	
Bacteroidia	1	0	0	0	
Betaproteobacteria	5	0	2	0	3
Deltaproteobacteria	3	1	1	0	2
Flavobacteria	4	0	1	0	1 & 2
Gammaproteobacteria	7	0	3	0	2
Lentisphaeria	0	0	1	0	
Planctomycetacia	0	0	1	0	
Planctomycetes	0	0	1	0	
Sphingobacteria	2	0	0	0	
Verrucomicrobia	0	0	1	0	

The most compelling and defining characteristic of the PCA groupings is that libraries within a group contain bacterial phlotypes that are more closely related within the group, than they are to bacteria in other groups. Figure 12 shows that the observed PCA groups can be recreated almost entirely based on the phylogenetic relatedness of their bacterial assemblages.

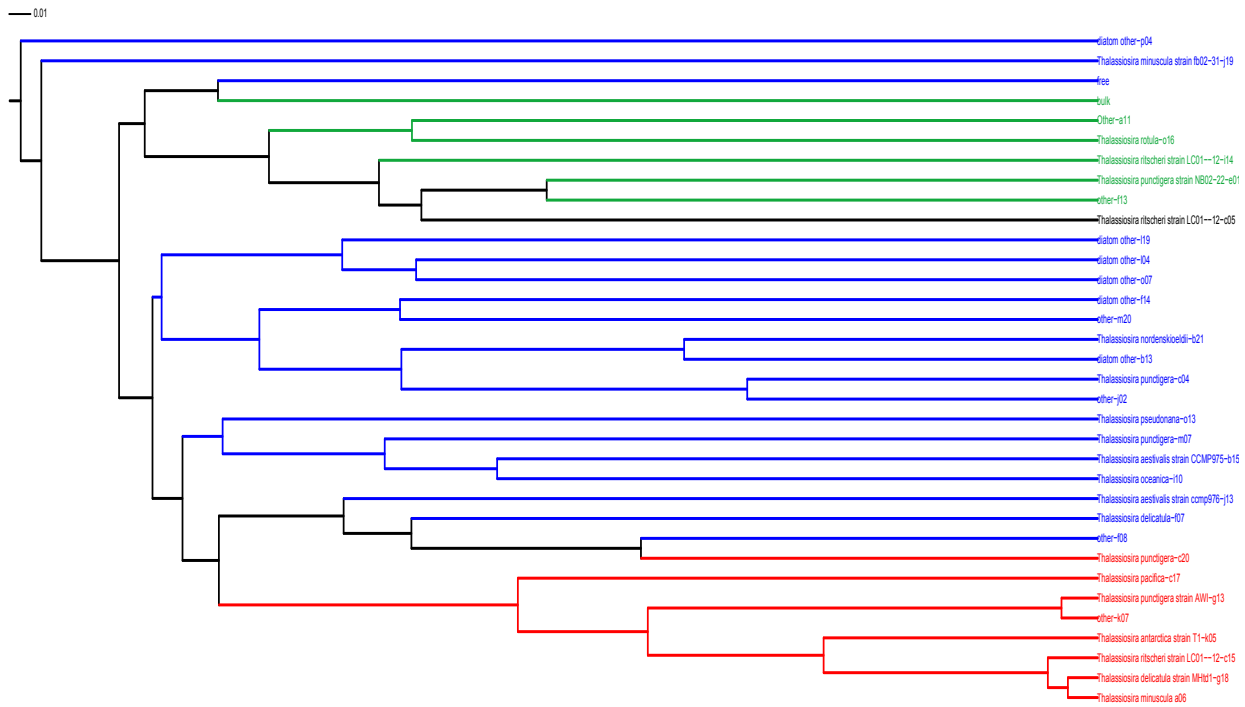


Figure 12. Clustering of libraries based on phylogenetic relatedness of the bacterial phylotypes they contain, calculated via a jackknife resampling procedure using the Unifrac metric. Blue = PCA group 1, red = PCA group 2, green = PCA group 3, black = a single host cell (c5) that did not fall into groups 1-3. The library groups defined by PCA (Figure 11) can be re-created almost entirely, based on phylogenetic relatedness of bacteria in the libraries.

The PCA-defined groups can be understood more readily by examining the phylogenetic tree on which the groups are based. The phylogenetic tree is depicted in Figure 13, with bacterial classes identified by color code. Figure 14 shows the same sequences in the same tree, but color-coded according to the PCA group in which each sequence appeared. Most phylotypes are distributed between PCA group 1 and PCA group 3, which include multiple proteobacterial groups (*Alpha*-, *Beta*-, and *Gammaproteobacteria*) as well as other bacterial classes. Although both groups include other members of the *Actinobacteria*, neither includes *Arthrobacter*, whereas PCA group 2 libraries are composed almost entirely of *Arthrobacter* phylotypes. PCA group 3 is distinguished from PCA group 1 by the absence of *Flavobacteria* and *Sphingobacteria* from PCA group 3. Several small clusters of closely related bacterial phylotypes appear to be present predominantly in only one PCA group (Figure 14).

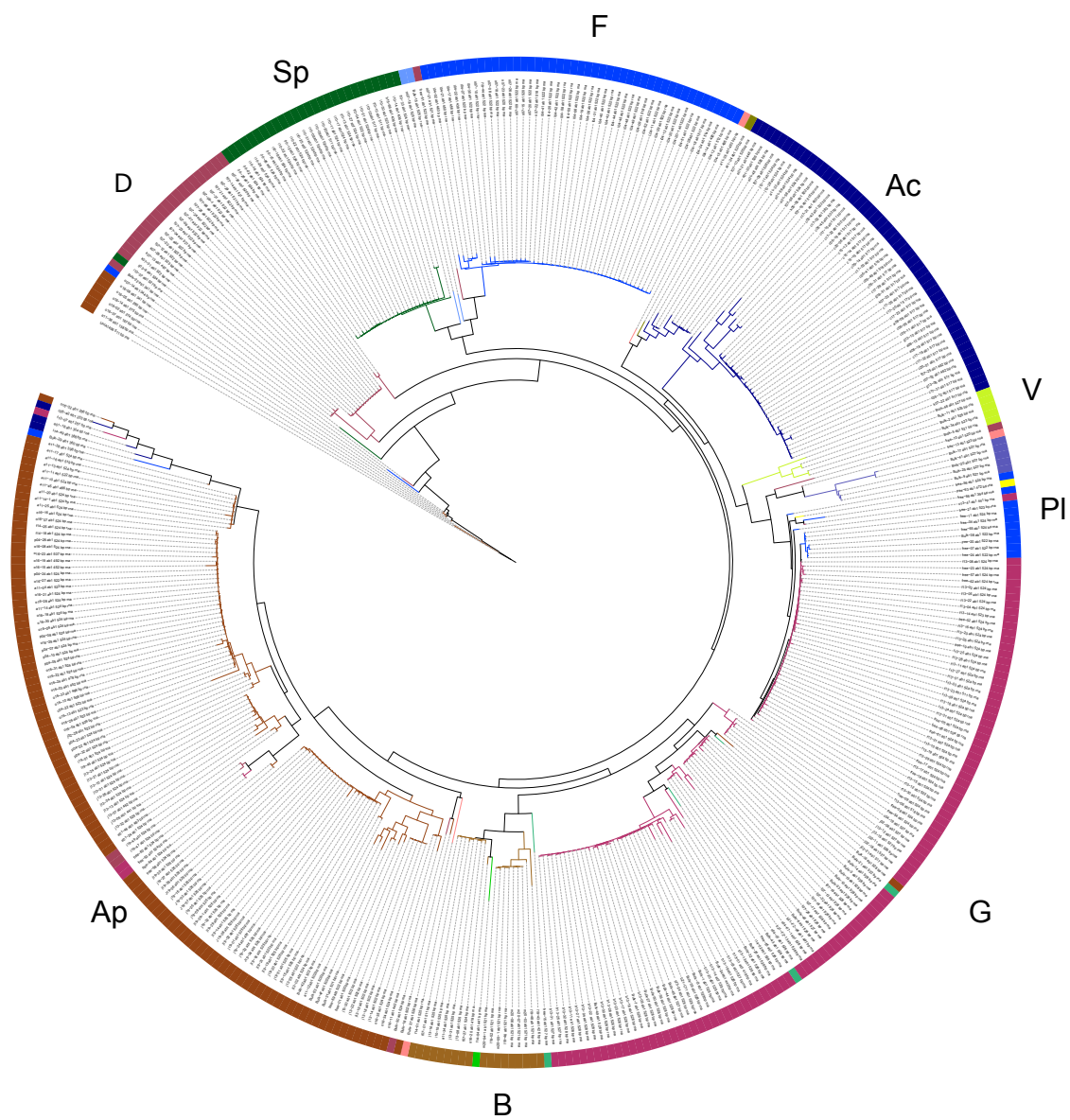


Figure 13. The phylogenetic tree used in analyses, in a representation produced by the Interactive Tree of Life (<http://itol.embl.de/>). Bacterial classes are identified by color, demonstrating satisfactory placement of sequences within their identified classes. Ac = Actinobacteria, Ap= Alphaproteobacteria, B= Betaproteobacteria, D= Deltaproteobacteria, F= Flavobacteria, G= Gammaproteobacteria, PI= Planctomycetacia, Sp= Sphingobactereia, V= Verrucomicrobiae.

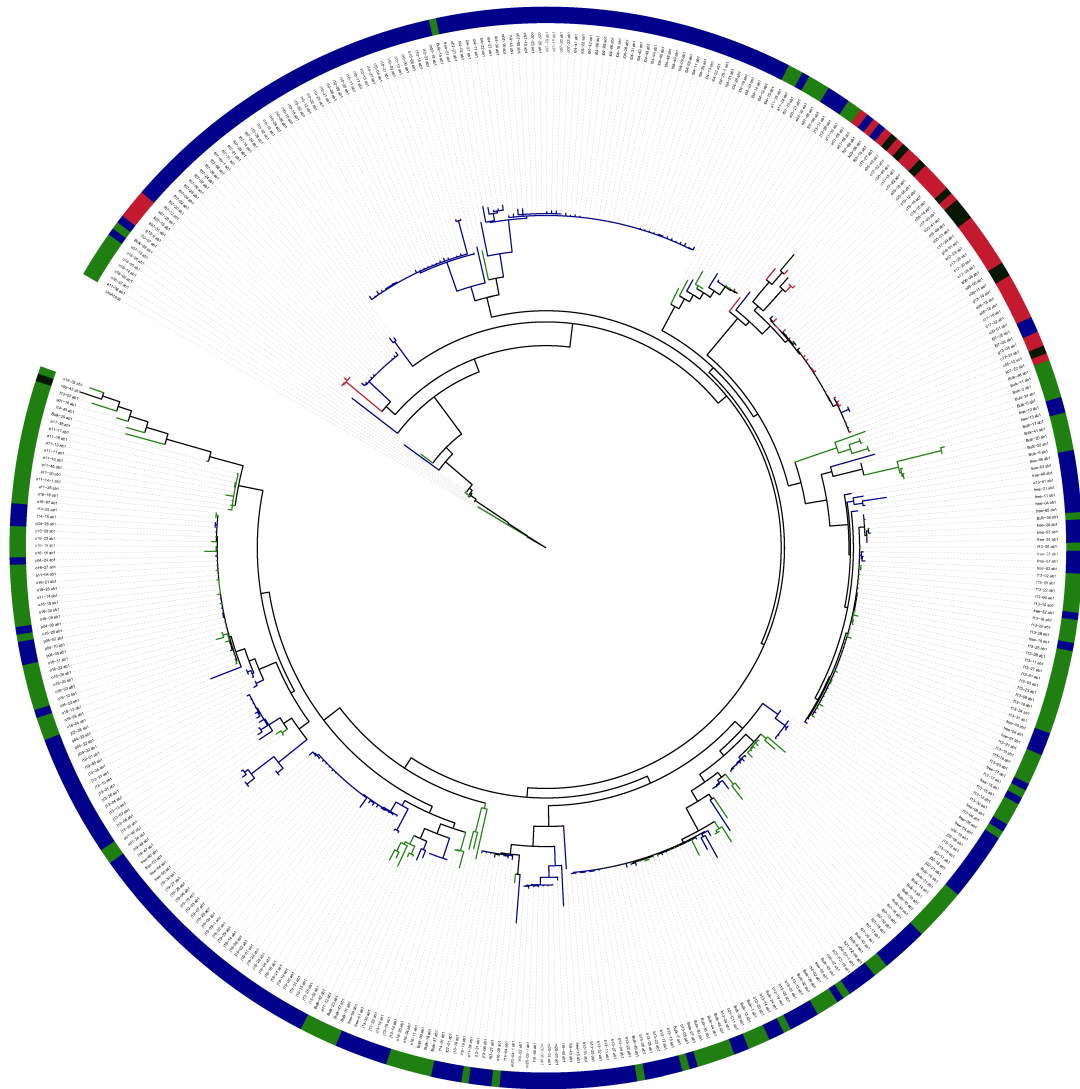


Figure 14. The same phylogenetic tree as represented in Figure 13, now color-coded according to the PCA group in which each sequence appeared. Blue = group 1, red = Group 2, green = Group 3. Black denotes sequences associated with one host cell library (c05--*Thalassiosira ritscheri* strain LC01--12) that was distinctly different from the three groups.

## DISCUSSION

In this preliminary study, *Thalassiosira*-associated bacterial assemblages were compared to those associated with other diatoms and other eukaryotes, and with the free-living bacteria and particle-associated bacterial assemblages recovered from the same water samples. Because

this study was exploratory in nature, no attempt was made to manipulate or pre-determine the host species present in the field sample. I did not attempt to make the sampling representative of the total host populations nor representative of the diatom populations most often seen. My data set predominantly consists of a well-studied diatom genus (*Thalassiosira*), and therefore is well suited for comparing bacterial assemblages among closely related host cells. I was also able to assess whether bacteria associated with diatoms are similar to those on the other organisms collected (i.e. flagellates, coccolithophores, and dinoflagellates) or to the free-living and particle-associated bacterial assemblages, and could test whether observed similarities or differences were related to host cell phylogeny.

The unusual nature of the sample material, i.e. bacterial 16S rDNA in the presence of abundant host-derived plastid 16S rDNA, required a novel amplification strategy. Using a 16S rDNA primer developed for a very different environmental context (Hodkinson and Lutzoni, 2009) a majority of the sequences I recovered were identified as bacterial rather than plastid in origin. These results confirm the selectivity of the 895F primer as reported by Hodkinson and Lutzoni (2009). Although the primer discriminates against plastid sequences, it did amplify mitochondrial 16S rDNA and other eukaryotic sequences (38% and 5.6% of total sequence effort, respectively). While I was unable to get 100% discrimination against plastid and other non-targeted sequences, this was not necessary. The developed methodology allowed me to substantially increase the number of utilizable bacterial sequences per effort.

Host cell libraries contained from 1 to 20 different bacterial phylotypes, with the majority of host cells containing more than one bacterial phylotype. The number of different phylotypes in a host-cell library is a minimum estimate of the number of bacterial cells that occurred on the host cell, but with the methods used there is no direct evidence for how many bacterial cells were attached to each host cell. While each cell was given equal sequencing effort, the 895F primer

does not amplify all bacterial groups equally and the sequencing effort was not exhaustive. Consequently I am unable to state with certainty that further sequencing effort applied to the same sample would not result in the discovery of more bacterial phylotypes.

Some of the bacteria found associated with host cells are comparable to those found in published lab-based studies. At the level of prokaryotic classes, my results are very similar to previous work done by Grossart et al. (2005); e.g. diatoms hosted a variety of *Flavobacteria* and *Sphingobacteria* as well as various *Alpha*- and *Gammaproteobacteria* (the latter two were found on non-diatom hosts as well). As noted earlier, *Flavobacteria* and *Sphingobacteria* are likely to be underrepresented in libraries created with the 895F primer, and may have been more abundant in the original sample than I observed in libraries. *Vibrio* were found on diatoms and particle-associated libraries, and have been noted in previous studies (Bidle and Azam, 2001). The consistency of my results with those previously reported suggests some similarities between culture-based data and those obtained from single-cell analysis of natural populations.

Although many of the bacterial orders and classes found in previous studies were observed in my sample, others found previously are missing. I did not find *Roseobacter* and members of the *Flexibacteriaceae* that were previously found in cultures of *Thalassiosira rotula*. That sample was from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton and was grown in Guillard's *f/2* or *f/10* medium (CCMP, Maine, USA) (Grossart et al., 2005); this is a much higher nutrient concentration than is typically seen at Station ALOHA. *Pseudoalteromonas* and *Alteromonas* have also been shown to be associated with diatoms (Bidle and Azam, 2001), but were not found in this study. *Campylobacter*, an *Epsilonproteobacteria* (Reimann et al., 2000), which was found associated with a dinoflagellate cell, and *Cytophagales* (Reimann et al., 2000; Bidle et al., 2003), which was found on a *Thalassiosira* host cell, were not found in the present study. Although the absence of *Flexibacteriaceae* might be attributed to primer bias, the

rest of the aforementioned groups are capable of being amplified by the 895F primer. Given that my sample includes similar diatom hosts, I suggest that these bacterial groups were not as prevalent in my sample as in the previously reported work. This may be due to differences in host-cell environment or in bacterial seed populations, but further study is needed to test this conjecture.

Only a few phylotypes appeared in either the free-living or the particle-associated libraries and in a host cell library. As noted earlier, previous studies have also commented on the differences between bacteria associated with host cells, and those present in the free-living and particle-associated bacterial assemblages. However, these two libraries fell within the same PCA groups and environmental clusters as the host cell libraries. Like other libraries in PCA groups 1 and 3, the free-living and particle-associated libraries included diverse bacteria drawn from multiple classes.

I observed cell-to-cell variations in the bacterial associates of diatoms and other eukaryotic hosts. *Thalassiosira*-derived libraries were the least likely to be significantly different in pairwise comparisons, which would argue for *Thalassiosira* cells sharing a characteristic bacterial assemblage. However, not all *Thalassiosira* cells were similar in their bacterial associates, and *Thalassiosira* appeared in all three PCA groups. One of the PCA groups consisted of host cells (primarily *Thalassiosira*) with *Arthrobacter* bacterial associates, which were absent from the other two PCA groups, and co-occurred with other bacteria in only 2 of the 34 libraries. The present data offer no insight into the possible functional causes or consequences of the observed differences in the bacterial assemblages attached to host cells. However, given the nearly complete separation of *Arthrobacter* from other bacteria on host cells, it is tempting to speculate that some bacteria may be able to colonize host cells to the exclusion of other bacteria.

The remaining two PCA groups were much more diverse in their bacterial assemblages, but were distinguished by the absence of *Sphingobacteria* and *Flavobacteria* from one PCA group and their presence in the other. The removal of *Flavobacteria* strongly affected PCA grouping. I suggest that *Actinobacteria* and *Flavobacteria* should be targeted for further study. It should be noted that removal of other bacterial classes also disturb PCA groups, demonstrating that these statistical groups are based on multivariate factors and not merely the presence or absence of just a few taxa.

## CONCLUSIONS

This study is among the first to examine patterns in bacterial assemblages attached to host cells at the single-cell level. Although unique assemblages could not be assigned to any one host-type, I found phylotypes (both *Arthrobacter*) that were predominantly associated with and shared by a subset of the *Thalassiosira* host cells. PCA and environmental clustering allowed the identification of three distinct groups of host cells based on similarities and differences in their associated bacteria. The most common host type, *Thalassiosira* spp., appeared in all three groups, and one group was comprised almost exclusively of *Thalassiosira* libraries. Other diatom and non-diatom hosts, and the free-living and particle-associated libraries were scattered among the groups. The placement of libraries in groups is explained by the phylogenetic relatedness of their component bacteria, which are more closely related within group than to bacteria in other groups. When *Actinobacteria* and *Flavobacteria* are removed from the principal coordinate analysis, groupings dissolve; however, the removal of other classes also results in disruption of PCA groups. This suggests that the PCA groupings depend on the presence of multiple classes of bacteria.



Based on the high degree of variation explained by PCA, I propose that analysis of a larger number of cells will provide further evidence for the existence of recurring patterns in diatom-bacteria associations, including recurring groups of recognizable bacterial assemblages. If true, more information will be needed to establish the functional basis of these groups. The next steps are to 1) expand upon my limited data set to better characterize patterns in diatom-associated bacterial assemblages, and 2) examine whether these assemblages operate as communities and more specifically, as components of a metaorganism.

## **FUTURE STUDIES**

My intent is to determine whether bacterial assemblages attached to a host cell are truly microbial communities *sensu* Clements (1916), who defined a community as a sort of metaorganism possessing “a well-defined level of organization with tight interactions among organisms that comprise a causal system and gives rise to emergent properties” (paraphrasing by Konopka, 2009). My long-term goal is to identify characteristic diatom-associated microbial communities, examine their functional interactions and understand their emergent properties.

My initial data set is only representative of one sample of water that happened to be dominated by one diatom genus. In the future, I will build upon my results by increasing the scope of the available data. I need to expand my data in two directions: additional diatom host species, and a range of environments. The expanded data set will enable me to assess the predictability of diatom-bacteria associations in diverse marine environments supporting a range of diatom host species. I also plan to refine the 895F primer for use on commonly seen marine bacteria, e.g. to include the *Flexibacteraceae* and provide better representation of the *Flavobacteria* and *Sphingobacteria*

I also intend to conduct an initial exploration of the functional relationships of bacteria within a defined assemblage, and their functional relationship(s) with the host cell. To truly address whether or not diatoms and bacteria are acting as a metaorganism, I must assess whether diatom-associated bacteria exhibit functional properties that might be expected of a close association between bacteria and host.

Appendix Table II lists several candidate genes that are relevant to an exploration of the functional role of diatom-associated bacteria. Genes that are relevant to a surface attached lifestyle include: enhancers of colanic acid production (algD), relatives of a polysaccharide intercellular adhesin expressed by *Staphylococcus* (PIA), RpoS-related sigma factors that are regulated by quorum sensing (RpoQ), and N-acyl-L-homoserine lactone synthetase-like proteins similar to those produced by *Dinoroseobacter* (luxI, lux R families) (Brenda et al., 2005; Miller et al., 2001). Genes that are relevant to the relationship of bacteria to their diatom host include: genes for vitamin B<sub>12</sub> production (Croft et al., 2005), and genes involved in degradation of the organic matrix on which the silica frustule of diatoms is constructed (Bidle et al., 1999).

It is likely that a metagenomic approach would be used, and Table II in the Appendix also lists genes that can function as scaffolding during genome assembly, or can be used as markers for separating the bacterial component of the metagenome from the host cell genome, as well as eukaryotic or prokaryotic viruses.

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APPENDIX. Table I- All sequences used in this study, the corresponding ARB-based identification, and a nearest neighbor that is a marine, freshwater, or soil inhabiting bacteria.

Sequence name	98% group name	ARB assigned ID	ACC	Autor and year	Collected from	Title	Publication (if given)
a06-06_ab1	44	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthroacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
a06-13_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthroacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
a06-18_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthroacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
a11-04_ab1	46	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventralina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
a11-10_ab1		Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Methylobacteriaceae_Methylobacterium	AB220086	Kim et al., 2006	freshwater	Culturable diversity of aerobic phototrophic bacteria from terrestrial and freshwater environments screened by PCR-amplification of the pufLM.	
a11-11_ab1	48	Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Methylobacteriaceae_Methylobacterium	AB220090	Kim et al., 2010	freshwater	Culturable diversity of aerobic phototrophic bacteria from terrestrial and freshwater environments screened by PCR-amplification of the pufLM.	
a11-12_ab1	49	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacteriales_Rhodobacteraceae_1/Phaeobacter_3	EF573869	Rojas-Jimenez et al., 2008	Site S25 near Coco's island	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
a11-13_ab1	33	Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Methylobacteriaceae_Methylobacterium	AB220086	Kim et al., 2006	freshwater	Culturable diversity of aerobic phototrophic bacteria from terrestrial and freshwater environments screened by PCR-amplification of the pufLM.	
a11-14_ab1	8	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	HM480263	Helms et al., 2010			
a11-14-1_a	33	Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Methylobacteriaceae_Methylobacterium	AB220087	Kim et al., 2007	freshwater	Culturable diversity of aerobic phototrophic bacteria from terrestrial and freshwater environments screened by PCR-amplification of the pufLM.	
a11-16_ab1	50	Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Methylobacteriaceae_Methylobacterium	AB220091	Kim et al., 2011	freshwater	Culturable diversity of aerobic phototrophic bacteria from terrestrial and freshwater environments screened by PCR-amplification of the pufLM.	
a11-17_ab1	33	Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Methylobacteriaceae_Methylobacterium	AB220088	Kim et al., 2008	freshwater	Culturable diversity of aerobic phototrophic bacteria from terrestrial and freshwater environments screened by PCR-amplification of the pufLM.	
a11-20_ab1	51	Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Methylobacteriaceae_Methylobacterium	AB220092	Kim et al., 2012	freshwater	Culturable diversity of aerobic phototrophic bacteria from terrestrial and freshwater environments screened by PCR-amplification of the pufLM.	
a11-21_ab1	15	Bacteria/Firmicutes_Bacilli/Bacillales_Staphylococaceae/Staphylococcus_1	EF188440	Portillo et al., 2008	Altamira cave seawater polluted by tunisian crude oil	Molecular characterization of total metabolically active bacterial communities of 'white colonization' in the Altamira cave.	Res. Microbiol. 0.0-0 (2008)
a11-24_ab1	52	Bacteria/Actinobacteria/Actinobacteria/Actinomycetales_Actinomycetaceae_2/uncultured	CU915110	Zrafi-Nouira et al., 2009	tunisian crude oil	Molecular diversity analysis and bacterial population dynamics of an adapted seawater microbiota during the degradation of Tunisian zanzarine oil.	Biodegradation 20:467-486 (2009)
a11-33_ab1	20	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Dermabacteraceae/Brachyacterium_1	AB101583	Morikawa et al., 2003	jellyfish	Characterization of a jellyfish-degrading bacterium isolate and its enzyme.	
a11-34_ab1	1	Bacteria/Proteobacteria/Betaproteobacteria/Burkholderiales/Comamonadaceae/Delftia	EU888308	Jorgensen et al., 2008	fresh water	Delftia lacustris sp. Nov: a peptidoglycan-degrading bacterium from fresh water . . .	Int. J. Syst. Microbiol. 59:2195-2199
a11-35_ab1	55	Bacteria/Actinobacteria/Actinobacteria/Propionibacteriales/Nocardioideae/Marmoricola	DQ448720	Gontang et al., 2004	marine sediments 15 m depth of South China Sea	Phylogenetic diversity of gram-positive bacteria cultured from marine sediments.	Appl. Environ. Microbiol. 72:3272-3282 (2007)
a11-36_ab1	56	Bacteria/Proteobacteria/Alphaproteobacteria/SAR11 clade/Deep 1	GQ377765	Ma et al., 2011			
a11-38_ab1	57	Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Methylobacteriaceae_Methylobacterium	AB220093	Kim et al., 2013			
a11-45_ab1	59	Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Methylobacteriaceae_Methylobacterium	AB220095	Kim et al., 2015			
b13-01_ab1	60	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062007	Zang et al., 2035	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-03_ab1	61	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062008	Zang et al., 2036	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-04_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061981	Zang et al., 2009	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-05_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061982	Zang et al., 2010	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-11_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061983	Zang et al., 2011	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-13_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061984	Zang et al., 2012	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-14_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061985	Zang et al., 2013	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-17_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061986	Zang et al., 2014	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-18_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061987	Zang et al., 2015	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-19_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061988	Zang et al., 2016	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-20_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061989	Zang et al., 2017	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-22_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061990	Zang et al., 2018	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-23_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061991	Zang et al., 2019	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-24_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061992	Zang et al., 2020	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-25_ab1	26	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062005	Zang et al., 2033	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-27_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061993	Zang et al., 2021	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-28_ab1	62	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062009	Zang et al., 2037	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-30_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061994	Zang et al., 2022	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-31_ab1	26	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062006	Zang et al., 2034	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b13-32_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061995	Zang et al., 2023	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b15-66_ab1	38	Bacteria/Bacteroidetes/Sphingobacteria_Sphingobacteriales_1/Sphingobacteriaceae/Sphingobacterium_1	AM085476	Zeng et al., 2011	deep sea sediment	Characterization of the bacterial diversity in a deep sea sediment core from the tropic western Pacific warm pool.	
b21-C11_ab	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061996	Zang et al., 2024	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
b21-FZ-06	63	Bacteria/Proteobacteria/Gammaproteobacteria_1/Alteromonadales_Alteromonadaceae	AB602430	Park et al., 2011	seawater	New species isolated from ocean.	
b21-FZ-13	64	Bacteria/Proteobacteria/Gammaproteobacteria_1/Alteromonadales_Alteromonadaceae	AB602430	Park et al., 2011	seawater	New species isolated from ocean.	



Sequence name	98% group name	ARB assigned ID	ACC	Autor and year	Collected from	Title	Publication (if given)
Bulk-1_ab1	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061997	Zang et al., 2025	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-10_ab	17	Bacteria/Proteobacteria/Gammaproteobacteria_1/Pseudomonadales_Pseudomonadaceae/Pseudomonas	AJ312173	Sikorski et al., 2001		Identification of complex composition; strong strain diversity and directional . . .	Environ. Microbiol.4:465-476 (2002)
Bulk-11_ab	65	Bacteria/Verrucomicrobia/Verrucomicrobiales_Verrucomicrobiales/Verrucomicrobiaceae_Persicirhabdus	EU236322	Sipkema et al., 2008		Microbial characterisation of Haliclona (?gellius) sp.: sponge and associated microorganisms.	Microb. Ecol. 58:903-920 (2009)
Bulk-13_ab	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061998	Zang et al., 2025	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-14_ab	17	Bacteria/Proteobacteria/Gammaproteobacteria_1/Pseudomonadales_Pseudomonadaceae/Pseudomonas	AJ312173	Sikorski et al., 2001		Identification of complex composition; strong strain diversity and directional . . .	Environ. Microbiol.4:465-476 (2002)
Bulk-15_ab	66	Bacteria/Bacteroidetes/Cytophagia_Cytophagales/Flammovirgaceae_1/Reichenbachella	DQ889876	Ranzer et al., 2006	Erythropodium caribaeorum	Phylogenetic diversity of bacteria associated with the natural product producing octocoral Erythropodium caribaeorum.	
Bulk-16_ab	67	Bacteria/Proteobacteria/Alphaproteobacteria/Sphingomonadales/Erythrobacteraceae/uncultured	GQ389018	Li et al., 2009	contaminated fresh water	Characterization of bacterial community structure in a drinking water distribution system during an occurrence of red water.	
Bulk-17_ab	43	Bacteria/Planctomycetes/Planctomycetacia_Planctomycetales_Planctomycetaceae/uncultured	HQ721409	Durbin et al., 2011	abyssal seawater	Microbial diversity and stratification of oligotrophic abyssal marine sediments at the southern edge of the South Pacific Gyre.	
Bulk-18_ab	68	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacteriales_Rhodobacteraceae_1/Sagittula	EU734592	Zheng et al., 2008	South China Sea	Mamelella alba gen. nov.; sp. Nov.; a marine bacterium of the Roseobacter clade in the order Rhodobacteriales.	
Bulk-19_ab	17	Bacteria/Proteobacteria/Gammaproteobacteria_1/Pseudomonadales_Pseudomonadaceae/Pseudomonas	AJ312173	Sikorski et al., 2001		Identification of complex composition; strong strain diversity and directional . . .	Environ. Microbiol.4:465-476 (2002)
Bulk-2_ab1	69	Bacteria/Verrucomicrobia/Verrucomicrobiales_Verrucomicrobiales/Verrucomicrobiaceae_Persicirhabdus	EU236322	Sipkema et al., 2008		Microbial characterisation of Haliclona (?gellius) sp.: sponge and associated microorganisms.	Microb. Ecol. 58:903-920 (2009)
Bulk-20_ab	70	Bacteria/Planctomycetes/Planctomycetacia_Planctomycetales_Planctomycetaceae/uncultured	EU236390	Sipkema et al., 2008	Haliclona cf. Gellius sp (marine sponge)	Bacterial characterisation of Haliclona (?gellius) sp.: sponge associated microorganisms.	Microb. Ecol. 58:903-920 (2009)
Bulk-21_ab	71	Bacteria/Proteobacteria/Gammaproteobacteria_Xanthomonadales_Xanthomonadaceae/Stenotrophomonas	EF471903	Zhu et al., 2007	antarctica	Isolation and screening of cytotoxic bacteria from Antarctica	
Bulk-22_ab	72	Bacteria/Proteobacteria/Deltaproteobacteria/Myxococcales/Myxococcaceae/Nannocystinae/Nannocystaceae	HM591449	Bae, 2010	seawater	Change in microbial community structure of seawater by microfiltration and filter systems.	
Bulk-23_ab	73	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacteriales_Rhodobacteraceae_1/Marinovum	GU474886	Pham et al., 2010	HOT-ALOHA	Time series-analysis of Monterey Bay coastal microbial picoplankton using a 'genome proxy' microarray	
Bulk-24_ab	74	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062010	Zang et al., 2038	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-25_ab	34	Bacteria/Planctomycetes/Planctomycetacia_Planctomycetales_Planctomycetaceae/uncultured	EU236390	Sipkema et al., 2008	Haliclona cf. Gellius sp (marine sponge)	Bacterial characterisation of Haliclona (?gellius) sp.: sponge associated microorganisms.	Microb. Ecol. 58:903-920 (2009)
Bulk-26_ab	37	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae/Vibrio_7	FJ457440	Gram et al., 2008	jelly fish	Antibacterial activity of marine culturable bacteria collected from a global sample of ocean surface waters and surface swabs of marine organisms	
Bulk-27_ab	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU061999	Zang et al., 2027	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-29_ab	75	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/Crocinitomix	EU050905	Tian et al., 2011	sediment from Kigs Bay, Artic	Bacterial, archaeal, and eukaryotic diversity in Arctic sediment as revealed by 16SrRNA and 18SrRNA gene clone libraries analysis.	Polar Biol. 32:93-103 (2009)
Bulk-3_ab1	17	Bacteria/Proteobacteria/Gammaproteobacteria_1/Pseudomonadales_Pseudomonadaceae/Pseudomonas	AJ312173	Sikorski et al., 2001		Identification of complex composition; strong strain diversity and directional . . .	Environ. Microbiol.4:465-476 (2002)
Bulk-30_ab	76	Bacteria/Proteobacteria/Deltaproteobacteria/Myxococcales/s/0319-020	FJ203334	Sunagawa et al., 2008	seawater (coral)	Bacterial diversity and White Plague Disease-associated community changes in the Caribbean coral Montastraea faveolata.	
Bulk-31_ab	77	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacteriales_Rhodobacteraceae_1/uncultured	EU799605	Shaw et al., 2008	Newport Harbour, RI	It's all relative: ranking the diversity of aquatic bacterial communities	
Bulk-32_ab	37	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae/Vibrio_7	FJ457440	Gram et al., 2009	jelly fish	Antibacterial activity of marine culturable bacteria collected from a global sample of ocean surface waters and surface swabs of marine organisms	
Bulk-33_ab	17	Bacteria/Proteobacteria/Gammaproteobacteria_1/Pseudomonadales_Pseudomonadaceae/Pseudomonas	AJ312173	Sikorski et al., 2001		Identification of complex composition; strong strain diversity and directional . . .	Environ. Microbiol.4:465-476 (2002)
Bulk-34_ab	78	Bacteria/Verrucomicrobia/Verrucomicrobiales_Verrucomicrobiales/Rubritaleaceae_Rubritalea	FJ203294	Sunagawa et al., 2008	Montastraea faveolata	Threatened corals provide underexplored microbial habitats	ISME J 3:512-521 (2009)
Bulk-36_ab	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062000	Zang et al., 2028	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-37_ab	79	Bacteria/Lentisphaerae_Lentisphaeria/Victivallales_Victivallaceae/uncultured	EU050935	Tian et al., 2007	sediment from Kigs Bay, Artic	Bacterial, archaeal, and eukaryotic diversity in the Arctic sediment as revealed by 16S rRNA and 18S rRNA gene clone libraries analysis.	Polar Biol. 32:93-103 (2009)
Bulk-38_ab	22	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/NS7 marine group	EF572834	Rojas-Jimenez et al., 2007	Coco's Island site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
Bulk-39_ab	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062001	Zang et al., 2029	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-41_ab	43	Bacteria/Planctomycetes/Planctomycetacia_Planctomycetales_Planctomycetaceae/uncultured	HQ721409	Durbin et al., 2011	abyssal seawater	Microbial diversity and stratification of oligotrophic abyssal marine sediments at the southern edge of the South Pacific Gyre.	
Bulk-42_ab	80	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacteriales_Rhodobacteraceae_1/Halassococcus	GU176617	Case et al., 2009	marine sediments	Pathogens, disease and chemical defense of algae in a warming ocean.	
Bulk-43_ab	81	Bacteria/Proteobacteria/Gammaproteobacteria_2/Legionellales_Coxiellaceae_Coxiella	EU249954	Speck et al., 2007	seawater, Palmyra Atoll	Polyphasic description of bacteria diversity in Pocillopora meandrina at Palmyra atoll.	
Bulk-44_ab	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062002	Zang et al., 2030	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-45_ab	82	Bacteria/Proteobacteria/Gammaproteobacteria_1/Alteromonadales_Alteromonadaceae/BD1-7 clade	EF575144	Rojas-Jimenez et al., 2008	site S25 near Coco's island	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
Bulk-46_ab	83	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062011	Zang et al., 2039	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-47_ab	84	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacteriales_Rhodobacteraceae_1/uncultured	EU799605	Shaw et al., 2008	Newport Harbour, RI	It's all relative: ranking the diversity of aquatic bacterial communities	
Bulk-48_ab	85	Bacteria/Verrucomicrobia/Verrucomicrobiales_Verrucomicrobiales/DEV007	GQ249493	Koehling et al., 2009	marine sediment	Microbial community composition of the anaerobic marine sediments in the Bay of Cadiz (Spain).	
Bulk-49_ab	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062003	Zang et al., 2031	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-5_ab1	86	Bacteria/Verrucomicrobia/Verrucomicrobiales_Verrucomicrobiales/Rubritaleaceae_Rubritalea	FJ203294	Sunagawa et al., 2008	Montastraea faveolata	Threatened corals provide underexplored microbial habitats	ISME J 3:512-521 (2009)
Bulk-50_ab	6	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062004	Zang et al., 2032	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-6_ab1	87	Bacteria/Proteobacteria/Gammaproteobacteria_1/Alteromonadales_Alteromonadaceae/BD1-7 clade	HQ163611	Walsh et al., 2008	Saaniche Inlet, 120 m depth	Microbial community genomics of a coastal dead zone	
Bulk-7_ab1	88	Bacteria/Proteobacteria/Gammaproteobacteria_1/Vibrionales_Vibrionaceae	GU062012	Zang et al., 2040	South China Seas	Phylogenetic diversity of bacterial communities in South China Sea mesoscale cyclonic eddy perturbations	
Bulk-9_ab1	34	Bacteria/Planctomycetes/Planctomycetacia_Planctomycetales_Planctomycetaceae/uncultured	EU236390	Sipkema et al., 2008	Haliclona cf. Gellius sp (marine sponge)	Bacterial characterisation of Haliclona (?gellius) sp.: sponge associated microorganisms.	Microb. Ecol. 58:903-920 (2009)
c04-07_ab1	89	Bacteria/Proteobacteria/Gammaproteobacteria_1/Alteromonadales_Alteromonadaceae	AB602430	Park et al., 2011	seawater	New species isolated from ocean.	
c04-07-1_a	39	Bacteria/Proteobacteria/Gammaproteobacteria_1/Alteromonadales_Alteromonadaceae	AB602430	Park et al., 2011	seawater	New species isolated from ocean.	

Sequence name	98% group name	ARB assigned ID	ACC	Autor and year	Collected from	Title	Publication (if given)
c04-19_ab1	91	Bacteria/Proteobacteria/Gammaproteobacteria_1/Pseudomonadales_Moraxellaceae/Acinetobacter	AY345462	Donachie et al., 2016	Lake Kauhako	Microbial communities in the Hawaiian archipelago: a microbial diversity hotspot	
c04-22_ab1	92	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
c05-05_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c05-11_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c05-12_ab1	23	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c05-14_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c05-19_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c05-31_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c05-41_ab1	25	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c05-42_ab1	94	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c05-43_ab1	95	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c05-45_ab1	96	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c05-49_ab1	25	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c15-12_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c15-14_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c15-15_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-02_ab1	97	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-03_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-15_ab1	98	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-18_ab1	23	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-20_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-21_ab1	99	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-22_ab1	100	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-23_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-25_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-29_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-31_ab1	23	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c17-32_ab1	23	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c20-01_ab1	23	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
c20-06_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
e01-18_ab1	103	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Kocuria_2	HM245634	Shubenkov et al., 2010	pockmark sediments, Baltic sea	Microbial community of reduced pockmark sediments (Gdansk Deep, Baltic Sea).	Microbiology 79:799-808
e01-21_ab1	104	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Kocuria_2	HM245634	Shubenkov et al., 2010	pockmark sediments, Baltic sea	Microbial community of reduced pockmark sediments (Gdansk Deep, Baltic Sea).	Microbiology 79:799-808
e01-24_ab1	105	Bacteria/Proteobacteria/Alphaproteobacteria/Sphingomonadales/Sphingomonadaceae_Sphingomonas_2	AB377219	Wantanabe et al., 2008	Nabeta bay, Japan	Isolation of marine bacterioplankton	
e01-30_ab1	27	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Kocuria_2	HM245634	Shubenkov et al., 2010	pockmark sediments, Baltic sea	Microbial community of reduced pockmark sediments (Gdansk Deep, Baltic Sea).	Microbiology 79:799-808
e01-46_ab1	107	Bacteria/Proteobacteria/Alphaproteobacteria/Sphingomonadales/Sphingomonadaceae_Sphingomonas_2	AB377219	Wantanabe et al., 2008	Nabeta bay, Japan	Isolation of marine bacterioplankton	
e01-48_ab1	27	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Kocuria_2	HM245634	Shubenkov et al., 2010	pockmark sediments, Baltic sea	Microbial community of reduced pockmark sediments (Gdansk Deep, Baltic Sea).	Microbiology 79:799-808
e01-58_ab1	20	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Dermabacteraceae/Brachybacterium_1	AB101583	Morikawa et al., 2003	jellyfish	Characterization of a jellyfish-degrading bacterium isolate and its enzyme.	
e01-59_ab1	20	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Dermabacteraceae/Brachybacterium_1	AB101583	Morikawa et al., 2003	jellyfish	Characterization of a jellyfish-degrading bacterium isolate and its enzyme.	
f07-01_ab1	108	Bacteria/Proteobacteria/Deltaproteobacteria/Burkholderiales/Comamonadaceae/Delftia	EU888308	Jorgensen et al., 2008	fresh water	<i>Delftia lacustris</i> sp. Nov; a peptidoglycan-degrading bacterium from fresh water . . .	Int. J. Syst. Microbiol. 59:2195-2199
f07-02_ab1	32	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
f07-04_ab1	111	Bacteria/Proteobacteria/Deltaproteobacteria/Myxococcales/Nannocystineae/Nannocystaceae	HM591449	Bae, 2010	seawater	Change in microbial community structure of seawater by microfiltration and filter systems.	
f07-06_ab1	2	Bacteria/Proteobacteria/Deltaproteobacteria/Myxococcales/Nannocystineae/Nannocystaceae	HM591449	Bae, 2010	seawater	Change in microbial community structure of seawater by microfiltration and filter systems.	
f07-07_ab1	2	Bacteria/Proteobacteria/Deltaproteobacteria/Myxococcales/Nannocystineae/Nannocystaceae	HM591449	Bae, 2010	seawater	Change in microbial community structure of seawater by microfiltration and filter systems.	
f07-09_ab1	113	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Micrococcus_1	HM640435	Ennore Traverses, near seashre	Kannan et al., 2010	Marine microbial diversity.	
f07-09-1_a	2	Bacteria/Proteobacteria/Deltaproteobacteria/Myxococcales/Nannocystineae/Nannocystaceae	HM591449	Bae, 2010	seawater	Change in microbial community structure of seawater by microfiltration and filter systems.	
f07-10_ab1	114	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_4/Intrasporangiaceae_1/Janibacter	DQ060381	Yu et al., 2005	arctic marine sediment	Isolation and Diversity of Actinomycetes in the Arctic Ocean Marine Sediments.	

Sequence name	98% group name	ARB assigned ID	ACC	Autor and year	Collected from	Title	Publication (if given)
f13-26_ab1	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
f13-27_ab1	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
f13-28_ab1	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
f13-29_ab1	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
f13-30_ab1	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
f13-31_ab1	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
f14-01_ab1	1	Bacteria/Proteobacteria/Betaproteobacteria/Burkholderiales/Comamonadaceae/Delftia	EU888308	Jorgensen et al., 2008	fresh water	<i>Delftia lacustris</i> sp. Nov; a peptidoglycan-degrading bacterium from fresh water...	Int. J. Syst. Microbiol. 59:2195-2199
f14-04_ab1	133	Bacteria/Firmicutes_Bacilli/Bacillales_Planococcaceae_2/uncultured	DQ310745	Hullar et al., 2005	stream	Recurring seasonal dynamics of microbial communities in stream habitats.	
f14-18_ab1	8	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
f14-25_ab1	8	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
free-01_ab	137	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacteriales_Rhodobacteraceae_1/uncultured	M63810	Britschgi et al., 2011	Sargasso Sea	Bacterioplankton population by rRNA gene cloning and sequencing.	Appl. Env. Micro. 57: 1706-1713
free-04_ab	10	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/NS7 marine group	EF572834	Rojas-Jimenez et al., 2007	Coco's Island site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
free-05_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-06_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-07_ab	22	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/NS7 marine group	EF572834	Rojas-Jimenez et al., 2007	Coco's Island site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
free-08_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-09_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-10_ab	138	Bacteria/Proteobacteria/Deltaproteobacteria/Sh765B-TzT-29	HQ721406	Durban et al., 2010	abyssal seawater	Microbial diversity and stratification of oligotrophic abyssal marine sediments at the southern edge of the South Pacific Gyre.	
free-11_ab	10	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/NS7 marine group	EF572834	Rojas-Jimenez et al., 2007	Coco's Island site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
free-13_ab	139	Bacteria/Acidobacteria/Holophagae/Holophagales_Holophagaceae/marine group	EU803879	Shaw et al., 2008	Newport Harbour, RI	It's all relative: ranking the diversity of aquatic bacterial communities	
free-15_ab	140	Bacteria/Proteobacteria/Gammaproteobacteria_2/Salinisphaerales_Salinisphaeraceae/ZD0417 marine group	AY907786	Fuchs et al., 2005	Arabian sea	Molecular identification of picoplankton populations in contrasting waters of the Arabian sea.	
free-17_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-18_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-19_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-20_ab	22	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/NS7 marine group	EF572834	Rojas-Jimenez et al., 2007	Coco's Island site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
free-21_ab	141	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/NS7 marine group	EF572834	Rojas-Jimenez et al., 2007	Coco's Island site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
free-23_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-24_ab	22	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/NS7 marine group	EF572834	Rojas-Jimenez et al., 2007	Coco's Island site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
free-51_ab	142	Bacteria/Proteobacteria/Deltaproteobacteria/SAR324 clade(Marine group B)	EU802560	Shaw et al., 2004	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	Environ. Microbiol. 10:2200-2210
free-52_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-53_ab	36	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/JL-ETNP-Y6	FJ825917	Min et al., 2007	filtered seawater at time of diatom bloom	Succession of bacterial community during spring diatom bloom in the Yellow Sea.	
free-54_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-55_ab	143	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-56_ab	144	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/NS7 marine group	EF572834	Rojas-Jimenez et al., 2007	Coco's Island site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
free-57_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-58_ab	9	Bacteria/Proteobacteria/Alphaproteobacteria/SAR11 clade/Surface 1	EF572324	Rojas-Jimenez et al., 2008	Coco Island Site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
free-59_ab	145	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacteriales_Rhodobacteraceae_1					
free-60_ab	36	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/JL-ETNP-Y6	FJ825917	Min et al., 2007	filtered seawater at time of diatom bloom	Succession of bacterial community during spring diatom bloom in the Yellow Sea.	
free-61_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-62_ab	10	Bacteria/Proteobacteria/Gammaproteobacteria_1/Oceanospirillales_3/SAR86 clade	EU803095	Shaw et al., 2008	Northeast of Colon, Panama	It's all relative: ranking the diversity of aquatic bacterial communities	
free-63_ab	146	Bacteria/Actinobacteria/Acidimicrobia_Acidimicrobiales/OCS155 marine group	AF382115	Fuchs et al., 2003	Atlantic	Bacterioplankton sorting during a transatlantic cruise	
free-64_ab	9	Bacteria/Proteobacteria/Alphaproteobacteria/SAR11 clade/Surface 1	EF572324	Rojas-Jimenez et al., 2008	Coco Island Site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
free-65_ab	10	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/NS7 marine group	EF572834	Rojas-Jimenez et al., 2007	Coco's Island site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
free-66_ab	147	Bacteria/Bacteroidetes/Flavobacteria_Flavobacteriales/Cryomorphaceae_2/NS7 marine group	EF572834	Rojas-Jimenez et al., 2007	Coco's Island site 23	Study of the diversity of marine microorganisms of Coco's Island based on environmental DNA samples	
g13-05_ab1	23	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	
g13-10_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococaceae/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on changes in bacterial community structure during algae outbreak in oligotrophic lakes.	

Sequence name	98% group name	ARB assigned ID	ACC	Autor and year	Collected from	Title	Publication (if given)
j13-13_ab1	29	Bacteria/Proteobacteria/Alphaproteobacteria/Sphingomonadales/Sphingomonadaceae_Sphingomonas_2	EU098003	Fuchs et al., 2007	Atlantic Meridional Transect cruise AMT6	Phylogenetic analysis of bacterioplankton inhabiting different oceanic provinces of the Atlantic Ocean.	
j13-14_ab1	7	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales_Rhodobacteraceae_1/Paracoccus_3	AM275338	Sivaji, 2006	deep sea sediment	Bacterial diversity in deep sea sediment of Indian Ocean.	
j13-15_ab1	11	Bacteria/Proteobacteria/Gammaproteobacteria_1/Pseudomonadales_Moraxellaceae/Acinetobacter	AY345460	Donachie et al., 2014	Lake Kauhako	Microbial communities in the Hawaiian archipelago: a microbial diversity hotspot	
j13-16_ab1	1	Bacteria/Proteobacteria/Betaproteobacteria/Burkholderiales/Comamonadaceae/Delftia	EU888308	Jorgensen et al., 2008	fresh water	Delftia lacustris sp. Nov: a peptidoglycan-degrading bacterium from fresh water . . .	Int. J. Syst. Microbiol. 59:2195-2199
j13-18_ab1	7	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales_Rhodobacteraceae_1/Paracoccus_3	AM275338	Sivaji, 2006	deep sea sediment	Bacterial diversity in deep sea sediment of Indian Ocean.	
j13-19_ab1	7	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales_Rhodobacteraceae_1/Paracoccus_3	AM275338	Sivaji, 2006	deep sea sediment	Bacterial diversity in deep sea sediment of Indian Ocean.	
j13-21_ab1	29	Bacteria/Proteobacteria/Alphaproteobacteria/Sphingomonadales/Sphingomonadaceae_Sphingomonas_2	EU098003	Fuchs et al., 2007	Atlantic Meridional Transect cruise AMT6	Phylogenetic analysis of bacterioplankton inhabiting different oceanic provinces of the Atlantic Ocean.	
j13-22_ab1	7	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales_Rhodobacteraceae_1/Paracoccus_3	AM275338	Sivaji, 2006	deep sea sediment	Bacterial diversity in deep sea sediment of Indian Ocean.	
j13-24_ab1	29	Bacteria/Proteobacteria/Alphaproteobacteria/Sphingomonadales/Sphingomonadaceae_Sphingomonas_2	EU098003	Fuchs et al., 2007	Atlantic Meridional Transect cruise AMT6	Phylogenetic analysis of bacterioplankton inhabiting different oceanic provinces of the Atlantic Ocean.	
j13-25_ab1	176	Bacteria/Proteobacteria/Alphaproteobacteria/Sphingomonadales	EF424402	Lal et al., 2007	soil	Novosphingobium panipatense sp. Nov. and Novosphingobium mathurense sp. Nov.; from oil-contaminated soil.	
j13-26_ab1	29	Bacteria/Proteobacteria/Alphaproteobacteria/Sphingomonadales/Sphingomonadaceae_Sphingomonas_2	EU098003	Fuchs et al., 2007	Atlantic Meridional Transect cruise AMT6	Phylogenetic analysis of bacterioplankton inhabiting different oceanic provinces of the Atlantic Ocean.	
j13-28_ab1	20	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Desulfurococcales/Brachybacterium_1	AB101583	Morikawa et al., 2003	jellyfish	Characterization of a jellyfish-degrading bacterium isolate and its enzyme.	
j13-30_ab1	7	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales_Rhodobacteraceae_1/Paracoccus_3	AM275338	Sivaji, 2006	deep sea sediment	Bacterial diversity in deep sea sediment of Indian Ocean.	
j13-31_ab1	1	Bacteria/Proteobacteria/Betaproteobacteria/Burkholderiales/Comamonadaceae/Delftia	EU888308	Jorgensen et al., 2008	fresh water	Delftia lacustris sp. Nov: a peptidoglycan-degrading bacterium from fresh water . . .	Int. J. Syst. Microbiol. 59:2195-2199
j13-32_ab1	179	Bacteria/Proteobacteria/Alphaproteobacteria/Sphingomonadales/Sphingomonadaceae_Sphingomonas_2	GQ388844	Li et al., 2009	contaminated fresh water	Characterization of bacterial community structure in a drinking water distribution system during an occurrence of red water.	
j19-01_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21259	Genoscope, 2008	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-02_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21260	Genoscope, 2009	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
J19-03_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21261	Genoscope, 2010	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
J19-04_ab1	180	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21276	Genoscope, 2025	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
J19-06_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21262	Genoscope, 2011	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
J19-07_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21263	Genoscope, 2012	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
J19-08_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21264	Genoscope, 2013	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-09_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21265	Genoscope, 2014	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-09-1_a	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21266	Genoscope, 2015	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-14_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21267	Genoscope, 2016	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-15_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21268	Genoscope, 2017	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-16_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21269	Genoscope, 2018	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-18_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21270	Genoscope, 2019	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-19_ab1	181	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21277	Genoscope, 2026	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-20_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21271	Genoscope, 2020	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-21_ab1	182	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21278	Genoscope, 2027	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-22_ab1	183	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21279	Genoscope, 2028	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-24_ab1	184	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21280	Genoscope, 2029	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-25_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21272	Genoscope, 2021	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-26_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Phyllobacteriaceae_1/Phyllobacterium	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
j19-27_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Phyllobacteriaceae_1/Phyllobacterium	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
j19-29_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21273	Genoscope, 2022	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-30_ab1	185	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Phyllobacteriaceae_1/Phyllobacterium	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
j19-31_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21274	Genoscope, 2023	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
j19-32_ab1	16	Bacteria/Proteobacteria/Alphaproteobacteria/Rhodobacterales/Bradyrhizobiaceae_Bosea_3	CU21275	Genoscope, 2024	sludge	Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge.	
k05-06_ab1	187	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococcales/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on charges in bacterial community structure during algae outbreak in oligotrophic lakes.	
k07-13_ab1	190	Bacteria/Proteobacteria/Deltaproteobacteria/Bdellovibrionales/Bacteriovoraceae/Peredibacter	AB369189	Masui et al., 2010	soil	Microbiological assessment of circulation mud fluids during the first operation of riser drilling by deep-earth. . .	
k07-22_ab1	23	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococcales/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on charges in bacterial community structure during algae outbreak in oligotrophic lakes.	
k07-23_ab1	3	Bacteria/Actinobacteria/Actinobacteria/Micrococcales_1/Micrococcales/Arthrobacter_3	GU305834	Chen et al., 2010	YangHe reservoir	Research on charges in bacterial community structure during algae outbreak in oligotrophic lakes.	
k07-26_ab1	191	Bacteria/Proteobacteria/Deltaproteobacteria/Bdellovibrionales/Bacteriovoraceae/Peredibacter	AB369189	Masui et al., 2010	soil	Microbiological assessment of circulation mud fluids during the first operation of riser drilling by deep-earth. . .	
k07-31_ab1	192	Bacteria/Proteobacteria/Deltaproteobacteria/Bdellovibrionales/Bacteriovoraceae/Peredibacter	AB369189	Masui et al., 2010	soil	Microbiological assessment of circulation mud fluids during the first operation of riser drilling by deep-earth. . .	

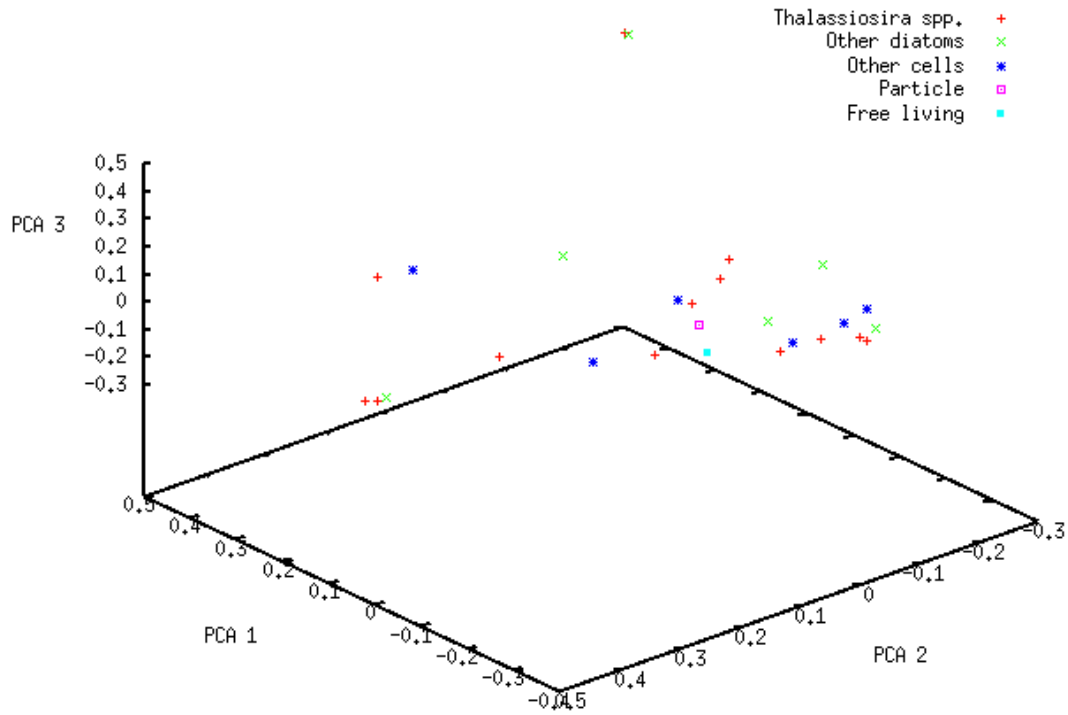




Sequence name	98% group name	ARB assigned ID	ACC	Autor and year	Collected from	Title	Publication (if given)
o16-33_ab1	242	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
o16-34_ab1	19	Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Bradyrhizobiaceae/Bradyrhizobium_2	AM936566	Milton et al., 2009	bioremediated soils	Bacterial community changes during bioremediation of aliphatic hydrocarbon-contaminated soil	
o16-35_ab1	19	Bacteria/Proteobacteria/Alphaproteobacteria/Rhizobiales_3/Bradyrhizobiaceae/Bradyrhizobium_2	AM936567	Milton et al., 2010	bioremediated soils	Bacterial community changes during bioremediation of aliphatic hydrocarbon-contaminated soil	
o16-37_ab1	243	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
p04-05_ab1	8	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
p04-07_ab1	8	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
p04-08_ab1	8	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
p04-10_ab1	8	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
p04-22_ab1	41	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae	GU472563	Bidre-Petit, 2010	Lake Pavin	Identification of sulfur-cycle prokaryotes in a low-sulfate lake (Lake Pavin) . . .	Micro. Ecology. 61: 313-327 (2011)
p04-23_ab1	41	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae	GU472563	Bidre-Petit, 2010	Lake Pavin	Identification of sulfur-cycle prokaryotes in a low-sulfate lake (Lake Pavin) . . .	Micro. Ecology. 61: 313-327 (2011)
p04-24_ab1	8	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
p04-28_ab1	30	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae/uncultured	GU118512	Sunagawa et al., 2009	Gorgonia ventalina (coral)	Threatened corals provide unexplored microbial habitat	PLoS One 5:E9554 (2010)
p04-32_ab1	41	Bacteria/Proteobacteria/Alphaproteobacteria/Caulobacteriales_Caulobacteraceae	GU472563	Bidre-Petit, 2010	Lake Pavin	Identification of sulfur-cycle prokaryotes in a low-sulfate lake (Lake Pavin) . . .	Micro. Ecology. 61: 313-327 (2011)

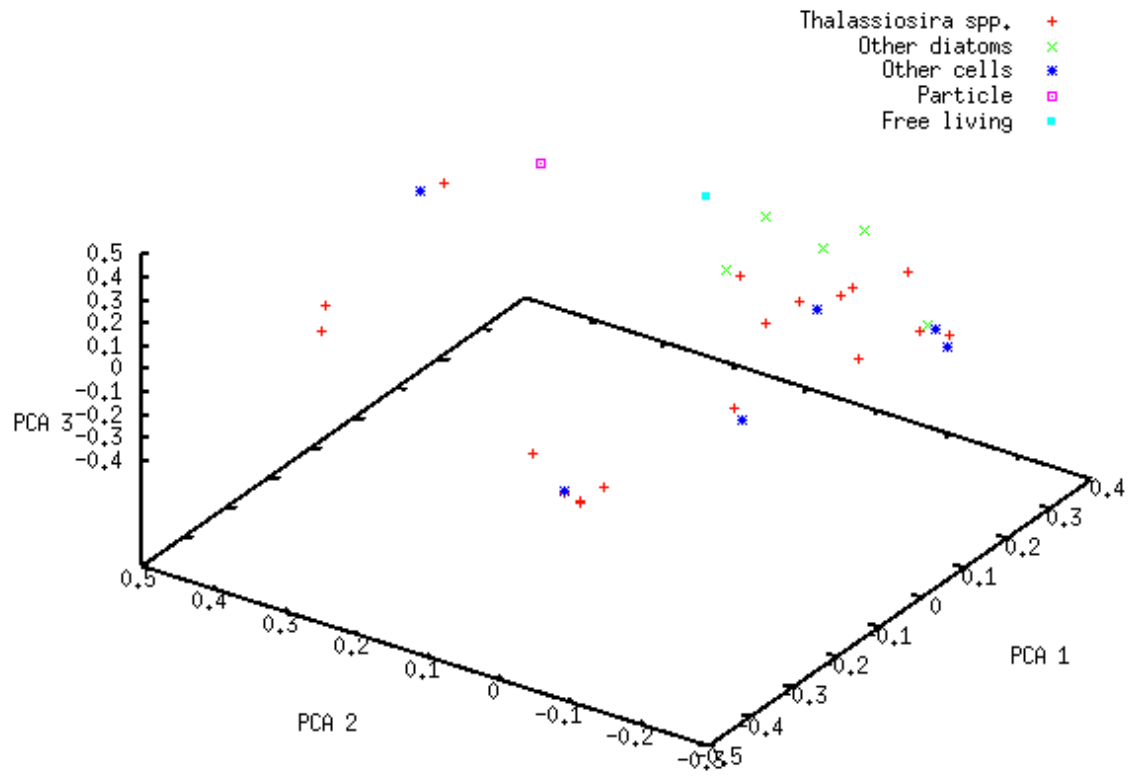
Figure I (A-F). Subtractive PCA analysis was used to examine the contribution of specific bacterial taxa to the PCA groups. The perspective best representing the positions of the groups are represented and are therefore not constant across the different tests.

A. Without Actinobacteria: Group 1 containing "Other diatoms" has dispersed. Most libraries in Group 2 are removed entirely.

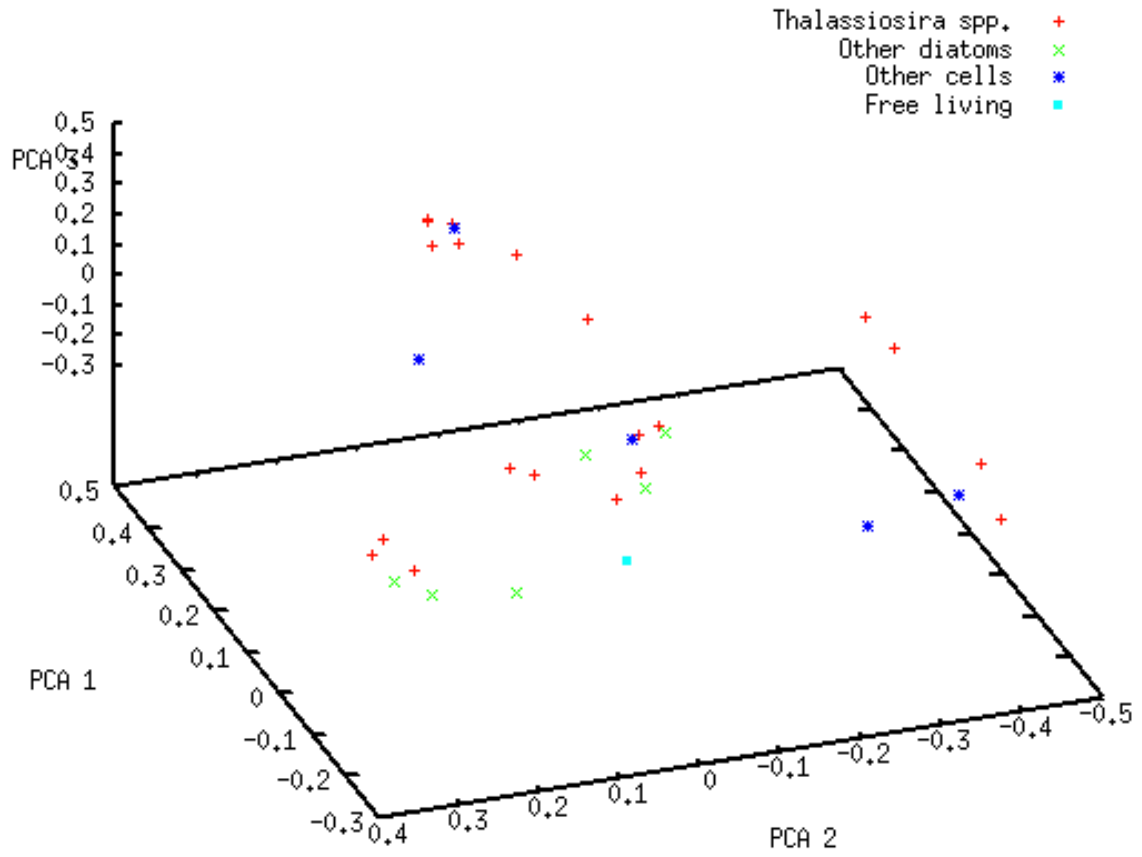




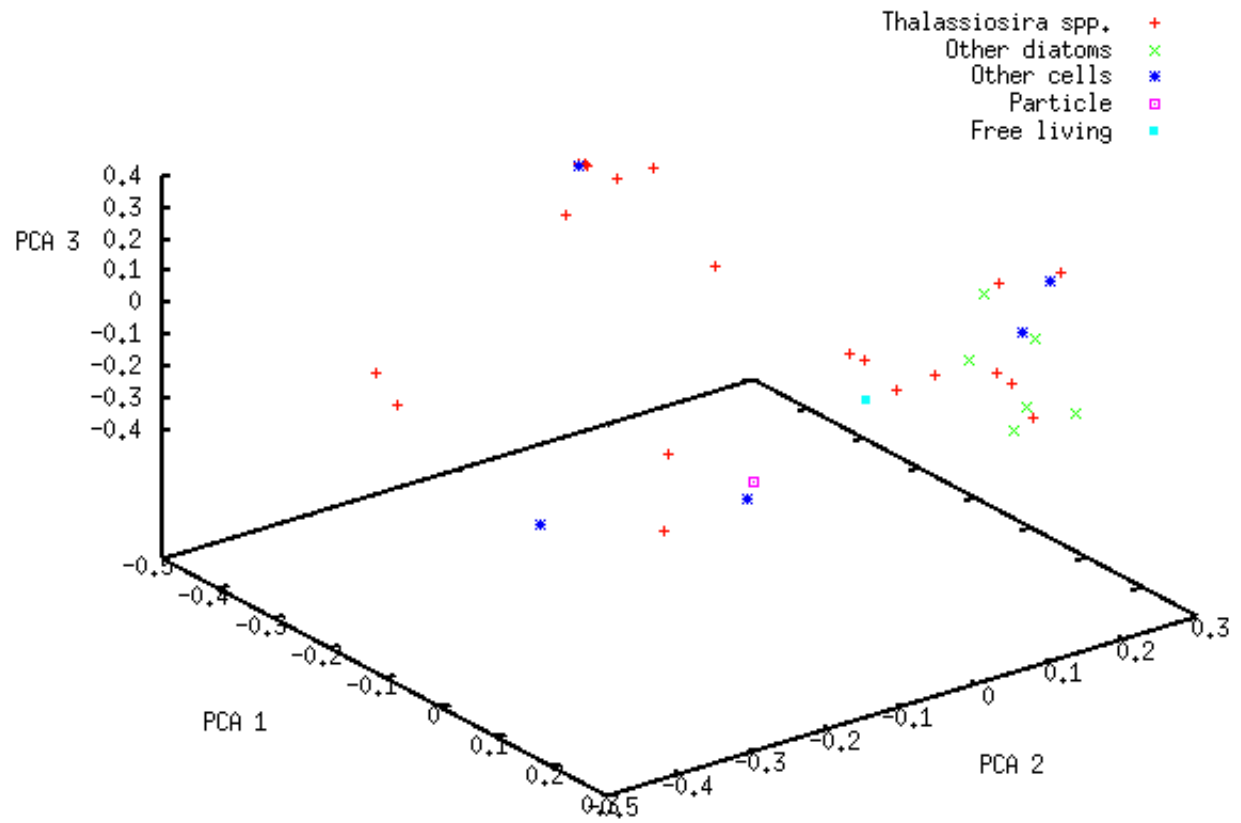
B. Without Alphaproteobacteria. Group 1 remains clustered, groups 2 and 3 disperse.



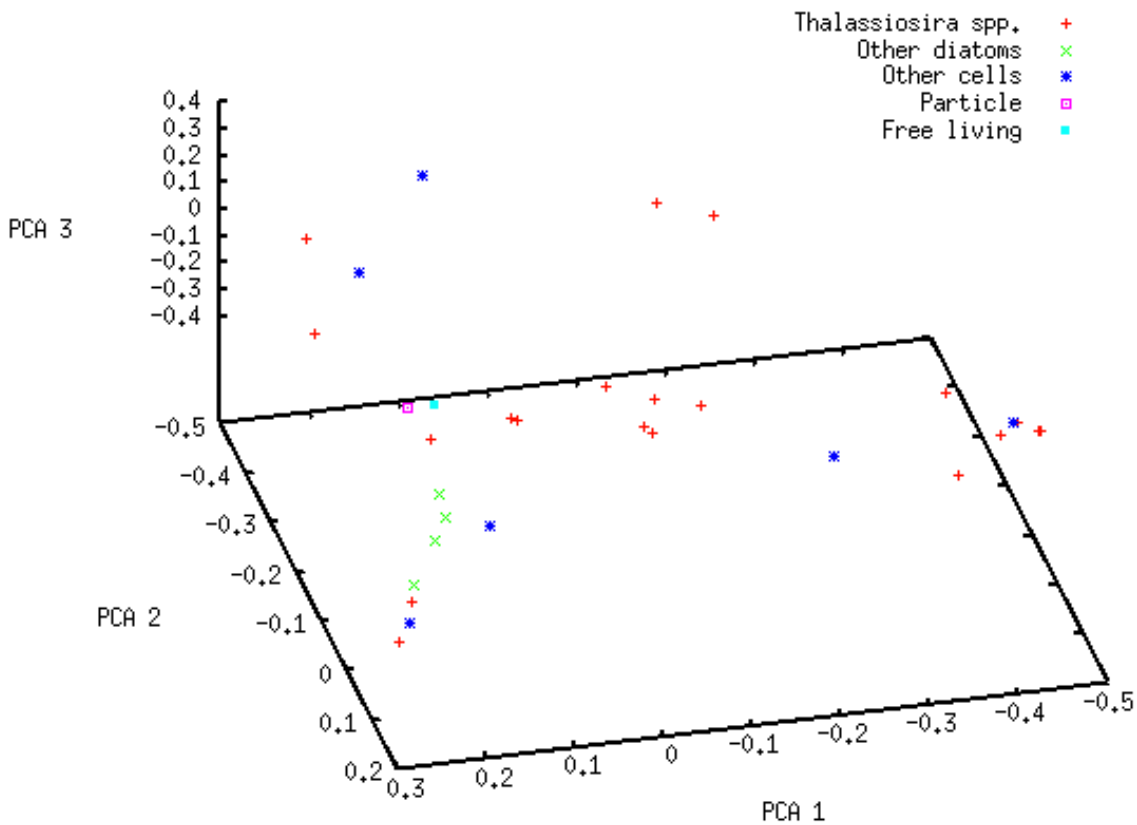
C. Without Betaproteobacteria. Most groups stay the same, Group 3 becomes more dispersed.



D. Without Deltaproteobacteria. Most groups stay the same, Group 2 becomes more dispersed.



E. Without Flavobacteria. Groups 1 and 3 merge, Group 2 remains.



F. Without Gammaproteobacteria. Group 1 and Group 3 remains the same, Group 2 shifts.

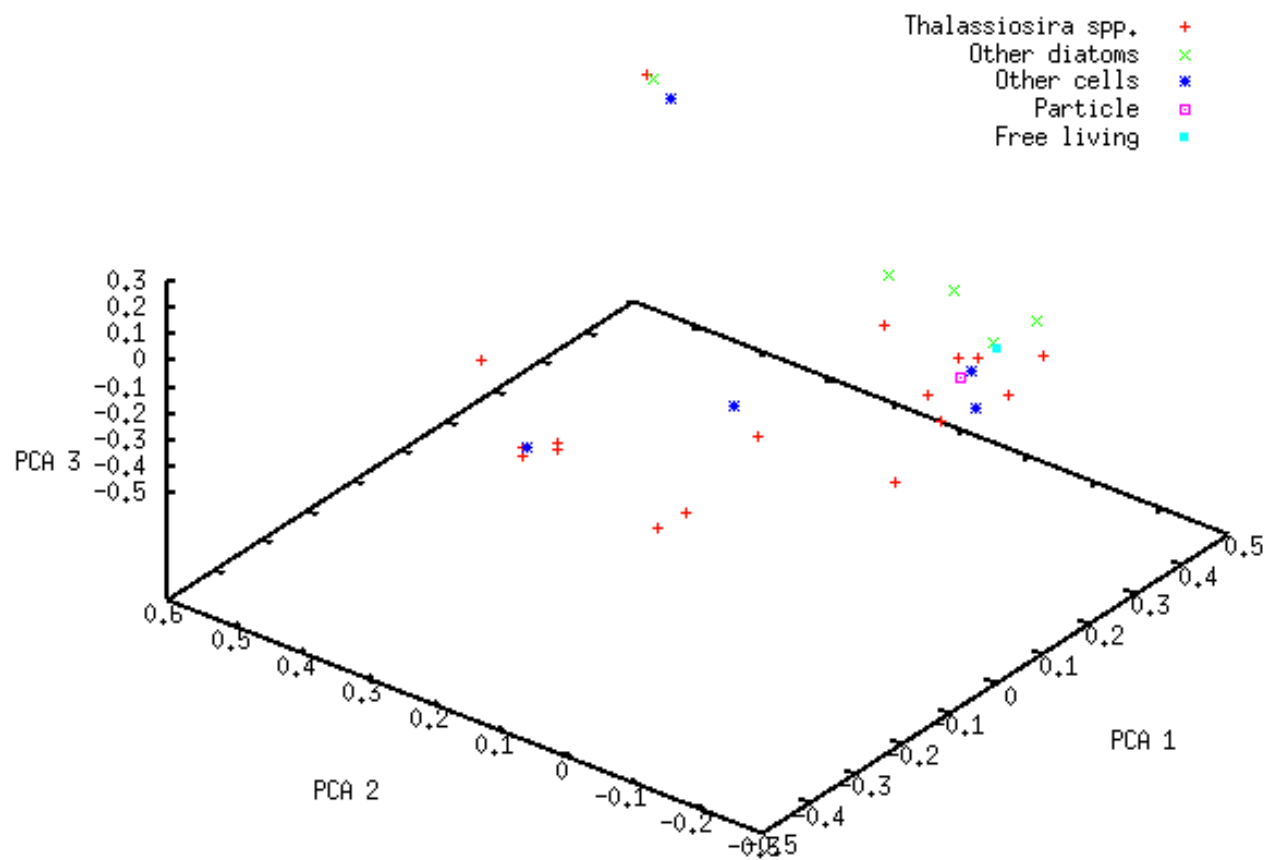


Table II: Suggested bacterial primers for further study.

General Function	Gene of interest	Bacterial ssp	Function	Entrez-ID	Publication
BIOFILM	PSM_A0203	<i>Pseudoalteromonas</i> sp.	biofilm formation protein	NC_014803.1	Qin et al, 2011
MOBILITY	flaF	<i>Caulobacter crescentus</i> CB15	flagellar biosynthesis regulatory protein	NC_002696.2	Schoenlein et al, 1990
	flagellar p-ring protein-like	<i>Phaeobacter</i> sp.	flagellar p-ring protein-like gene	EU414838	Slightom, 2008
	flgB	<i>Pseudoalteromonas atlantica</i>	lagellar basal body rod protein FlgG	NC_008228.1	JGI
ALGINATE	alginate lyase	<i>Halomonas marina</i>	alginate lyase	AB018795.1	Kraiwattanapong et al, 1999
	AlgI	<i>Hyphomonas neptunium</i>	alginate biosynthesis protein	NC_008358.1	Badger et al, 2006
ALTERNATIVE PHYLOGENETIC MARKER	dnaK	Various Alphaproteobacteria	molecular chaperone DnaK	NC_005027.1	Glockner et al, 2003
IDENTIFICATION GENES	GazF2/KEdtmR	Diatom specific	cox1	n/a	Evans et al, 2007
	895F	Bacteria specific	16S	n/a	Hodkinson et al, 2009
NUTRIENTS	CbiA/cobB	Alpha- and Gammaproteobacteria and Bacteroidetes	cobyrinic acid a,c-diamide synthase	NC_013716.1	Bertrand et al, 2011
ANTIBACTERIAL	VVM_01566	<i>Vibrio vulnificus</i>	isopenicillin N synthase	NC_014966.1	Park et al, 2011
	Cseg_3960	<i>Caulobacter segnis</i>	antibiotic biosynthesis monooxygenase	NC_014100.1	JGI
	HNE_0497	<i>Hyphomonas neptunium</i>	antibiotic biosynthesis monooxygenase domain-containing protein	NC_008358	Badger et al, 2006
	MADE_1018400	<i>Alteromonas macleodii</i>	multiple antibiotic resistance (MarC)-related protein	NC_011138.2	Ivars-Martinez et al, 2008
CHEMOTAXIS	cheA-like gene	<i>Phaeobacter</i> sp.	chemotaxis protein-like (cheA) gene	EU414830.1	Slightom, 2008
	CheW	<i>Alteromonas macleodii</i>	chemotaxis protein	NC_011138.2	Ivars-Martinez et al, 2008
OTHER	chiA	<i>Vibrio proteolyticus</i>	chitinase A precursor protein	chitinase A precursor protein	Itio et al, 2007
VIRUSES	orf-1, orf-2	<i>Rhizosolenia setigera</i> RNA virus	polyprotein, capsid proteins, complete cds	AB243297	Nagasaki, 2008
	complete genome	<i>Chaetoceros salsugineum</i> DNA virus	complete genome	NC_007193.2	Nagasaki et al, 2005
	complete genome	<i>Chaetoceros tenuissimus</i> DNA virus	complete genome	NC_014748.1	Nagasaki, 2008
	complete genome	<i>Flavobacterium</i> phage	complete genome	NC_006356	Borriss et al, 2007
	complete genome	<i>Roseophage</i> SI01	complete genome	AF189021.1	Rohwer, 2000