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Do Phycosphere associated bacteria affect the growth and lipid production of Phaeodactylum tricornutum

A. M. Chorazyczewski, P. V. Zimba, X. Mayali, J. Fox

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DO PHYCOSPHERE-ASSOCIATED BACTERIA AFFECT THE GROWTH AND LIPID PRODUCTION OF *PHAEODACTYLUM TRICORNUTUM* BOHLIN?

A Thesis

by

ADAM M. CHORAZYCZEWSKI

BS, University of California Santa Cruz, 2014

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTERS OF SCIENCE

in

FISHERIES AND MARICULTURE

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ADAM M. CHORAZYCZEWSKI

This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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August 2018

ABSTRACT

Microalgae biofuels are a promising alternative to fossil fuel energy. However current microalgal biofuel production is inefficient making it more expensive than fossil fuels. To make microalgal biofuels a realistic alternative energy source, improvements are needed in the production of microalgae biomass. Phycosphere-associated bacteria are known to influence microalgae growth and metabolite production. Understanding these interactions could provide new methods for enhancing microalgae biomass and improving the efficiency of producing microalgae biofuels. Co-cultures of *Phaeodactylum tricornutum* Bohlin and single bacterial species were compared to determine what impact phycosphere-associated bacteria can have on microalgae. Growth rates, total lipid accumulation and fatty acid profiles for each co-culture were compared to an axenic culture of *P. tricornutum* to assess the impact of the bacterial association. *Marinobacter* 3-2 significantly increased the lipid accumulation of *P. tricornutum* during early exponential growth. Two bacterial strains, *Algoriphagus* ARW1R1 and *Muricauda* ARW1Y1, significantly lowered the maximum growth rate and cell densities of *P. tricornutum* in early and late exponential growth. Lower cell densities in these co-cultures resulted in lower lipid accumulation. Our results suggest that co-culturing *P. tricornutum* with a phycosphere-associated bacterial species, *Marinobacter* 3-2, could help improve the efficiency of producing algal biomass and lipids.

DEDICATION

This work is dedicated to my late father, John Chorazyczewski.

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I. INTRODUCTION

The development of alternative sources of energy to petroleum is critical to meet our long term energy needs. Currently, biofuel production from lignocellulose is inefficient, expensive, and impractical as a replacement for fossil fuels (Christi 2007). The use of agricultural crops such as corn and soybeans for biofuel production exacerbates human food security concerns. Biofuels derived from algae are one promising option. Microalgae grow faster and produce more lipids than lignocellulose plants, making them a better source for biofuels production (Christi 2007, 2013, Do Nascimento et al. 2013). Additionally microalgae do not require arable land and therefore do not compete with traditional agricultural crops for farm land (Chauton et al. 2013, Andersson et al. 2014). Microalgal ponds can be filled with water not suitable for growing crops such as saline or gray water, reducing impacts on freshwater resources (Andersson et al. 2014). These physiological and environmental benefits make microalgal biomass a much more suitable source for biofuels than traditional oil crops.

Commercially grown microalgae can ultimately yield more products than just biofuels, including nutritional supplement and industrial products such as pigment's and glycerol (Christi 2007, Subashchandrabose et al. 2011, Steinrucken et al. 2018). Algal biomass can also be processed into feeds for agriculture/aquaculture (Subashchandrabose et al. 2011). Essential marine fatty acids provided by algae to humans as dietary supplements include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are synthesized by many marine microalgae species and accumulate in higher trophic levels (Brennan and Owende 2010, Muhlroth et al. 2013). Most of EPA and DHA consumed by humans originates in fish (Brennan and Owende 2010, Muhlroth et al. 2013). As the harvest of fish and other ocean organisms

cannot keep up with current human demand, new sources of EPA and DHA need to be developed (Steinrucken et al. 2018).

Long chain fatty acids, such as those found in microalgae, are most desirable for biofuels (Leyva et al. 2014). These fatty acids, such as EPA/DHA, are essential compounds only produced by algae, bacteria, or protozoans (Christi 2007, Lund et al. 2008). These fatty acids and the other commercial products from microalgae can enhance the value of algal biomass. However, the production of biofuels and other products from microalgae is still more costly and less efficient than producing these products from other sources, e.g. as refining EPA and DHA from fish oil (Christi 2007, 2013, Brennan and Owende 2010, Do Nascimento et al. 2013, Steinrucken et al. 2018).

There are several barriers to making the production of biofuel from microalgae cost efficient and competitive with petroleum. These issues can be categorized into three main problems: producing sufficient biomass for fuel production, cost and energy efficiency of harvesting that biomass, and the recovery of oil from the biomass (Chisti 2013, 2015, Vandamme et al. 2018). Increasing density of cell cultures and lipid accumulation in microalgal cultures could help mitigate some of the challenges with commercial microalgae production (Leyva et al. 2014, Han et al. 2016, Park et al. 2017). Increasing the lipid content and cell density of microalgal cultures may not completely solve all of the challenges facing commercial production, but it will make the processes of cell grow out and lipid extraction more efficient. Novel methods for increasing growth and cellular lipid content need to be developed in conjunction with improved techniques and technologies for harvesting and processing microalgae to make biofuels a realistic alternative to fossil fuels.

Bacteria have coexisted and evolved with microalgae for millions of years, developing mutualistic interactions that can increase microalgal growth and enhance the production of valuable products (Cole 1982, Ferrier et al. 2002, Croft et al. 2005, Amin et al. 2009, Seyedsayamdost et al. 2011, Amin et al. 2012, Do Nascimento et al. 2013, Buchan et al. 2014). Bacteria can positively impact microalgae through nutrient recycling and the production of vitamins and metabolites that stimulate algal growth (Cole 1982, Croft et al. 2005, Amin et al. 2012, Cooper and Smith 2015). Waste excreted by the algal cell can be recycled by bacteria (Amin et al. 2012). Interactions between bacteria and microalgae occur in a region surrounding the microalgal cell in which concentrations of microalgal exudates and bacterial metabolites are higher than found in the surrounding environment (Bell et al. 1972, Amin et al. 2012, Seymour et al. 2017). This area is termed the phycosphere and is conceptually similar to the rhizosphere around roots. Bacterial growth is stimulated by the excreted products of the algae, such as ammonia, and in return the algae benefit from the recycled nutrients and other metabolites, such as vitamins, produced by the bacteria (Bell et al. 1972, Amin et al. 2009, 2012). Bacterial metabolites can increase growth rates or lipid content in microalgae, which can make the production of biofuels more efficient and improve commercial microalgal biomass production (Chisti 2007).

Secondary metabolites from a variety of metabolic pathways can passively diffuse or be actively transported from marine bacteria to the phycosphere (Decho et al. 2011, Amin et al. 2012). Some of these bacterial metabolites can actively influence planktonic communities when they are released into the surrounding environment (Cole 1982, Seyedsayamdost et al. 2011, Paul et al. 2013). B vitamins, particularly B_{12}, are essential micronutrients for many microalgae and typically must be obtained from the environment (Haines and Guillard 1974, Croft et al. 2005,

Subashchandrabose et al. 2011). The concentration of cobalamin (vitamin B_{12}) found in sea water typically approximates 3 ng L^{-1}, which is below the concentration required for maximal microalgal growth (Croft et al. 2005). Vitamin B auxotrophic microalgae rely on phycosphere-associated bacteria as the main source of B vitamins, suggesting a mutualistic relationship where the bacteria synthesize B vitamins, stimulating microalgae growth that releases nutrients for the bacteria (Haines and Guillard 1974, Croft et al. 2005, Subashchandrabose et al. 2011). Bacterial metabolites other than vitamins have also been shown to impact microalgal growth and lipid profiles (Cole 1982, Seyedsayamdost et al. 2011, Paul et al. 2013). Seyedsayamdost et al. (2011) showed that *Phaeobacter gallaeciensis* produced auxins and antibiotics in the presence of a diatom bloom (*Emiliania huxleyi*). The auxins stimulated microalgal growth and the antibiotics prevented harmful bacteria from colonizing the diatoms (Seyedsayamdost et al. 2011). Maruyama et al. (1986) showed that some marine bacteria have the ability to produce cytokinin, a plant hormone known to promote growth in algae. Paul et al. (2013) reported an interaction between the bacterium, *Dinoroseobacter shibae*, and a diatom (*Thalassiosira pseudonana*) in which the production of intracellular amino acids within the diatom were upregulated. Chemical interactions between microalgae and bacteria are complex, involving numerous metabolites produced by both the bacteria and microalgae, dynamic interactions between metabolites, and the up and down regulation of different species (Cole 1982, Le Chavontan et al. 2013, Kim et al. 2014, Wang et al. 2014). The identification of bioactive metabolites that have a positive impact on microalgal growth and lipid content could improve commercial microalga culture and make biofuels and nutritional supplements more cost effective to produce.

Bacterial interactions that can increase the lipid content and alter the fatty acid profile of microalgae could enhance the efficiency of EPA production, thereby providing a more cost

effective and sustainable source of essential fatty acids (Do Nascimento et al. 2013). Microalgae having increased levels of EPA and other essential fatty acids could be utilized in the aquaculture industry as an alternative for fishmeal and fish oil (Brennan and Owende 2010, Natrah et al. 2014). The use of phycosphere-associated bacterial interaction to improve microalgal growth and lipid accumulation would be a novel method for improving the efficiency and cost effectiveness of commercial microalgae cultivation for both biofuels and other desirable products.

Some phycosphere-associated bacteria exhibit inhibitory interactions with their microalgal hosts (Cole 1982, Mayali et al. 2004, Seyedsayamdost 2011, Amin et al. 2012). Inhibitory effects of bacteria on microalgae occur through competition for nutrients and the release of bioactive metabolites (Cole 1982, Paul and Pohnert 2011, Manset et al. 2013, Amin et al. 2015, Han et al. 2016). Many studies have identified algicidal activity of marine bacteria and these interactions between microalgae and bacteria can play an important role in planktonic community structure and succession (Cole 1982, Doucette et al. 1999, Mayali and Azam 2004, Paul and Pohnert 2011, Seyedsayamdost et al. 2011). These inhibitory/algicidal interactions can either target specific species or affect a broad range of microalgae (Fukami et al. 1992, Imai et al. 1995). Paul and Pohnert (2011) showed that a marine bacterium (*Kordia algicida*) secreted a protease enzyme that had strong algicidal effects on three of the four marine diatoms tested. The non-specific nature of the algicidal activity observed suggests that the protease enzymes inhibit the growth of a wide range of microalgal species. Seyedsayamdost et al. (2011) identified a bacterium from the roseobacter clade producing a potent algicidal compound. In that study, *Phaeobacter gallaeciensis* would biosynthesize algicidal compounds in the presence of p-coumaric acid, a product of lignin breakdown that signals algal cell senescence. The effect of these compounds were specific to *Emiliania huxleyi* and *Rhodomonas salina*. The algicidal

effects required greater than a 15-fold higher concentration of algicidal compounds to have the same impact on other species tested (Seyedsayamdost et al. 2011). Specific interactions such as those observed by Seyedsayamdost could be utilized to target specific microalgal species without affecting the entire microalgae community. Understanding the complex nature of these interactions, how they affect the microalgal community assemblage, and identifying the specific metabolites involved may provide an effective technique to control harmful algae blooms (HABs).

HABs are becoming increasingly common due to rising sea temperatures and high nutrients loads in both fresh and coastal waters. The use of bacteria has been proposed as a natural solution to mitigate HABs (Doucette 1999, Mayali and Azam 2004, Paul and Pohnert 2011, Amin et al. 2015). Doucette (1999) showed that a gram negative bacterium isolated from sea water containing no detectable HAB species had strong algicidal activity on the dinoflagellate HAB (*Gymnodinium breve* = *Karenia brevis*). The same bacterium had no effect on a closely related non-toxic species of dinoflagellate. Doucette hypothesized that algicidal bacteria in the ocean are ubiquitous and can control the outbreak of HAB-forming species such as *G. breve.* Identifying these bacteria and how they interact with specific microalgae species is the next step in the fight against HABs.

The goal of this study was to improve the understanding of interactions between phycosphere-associated bacterial species and one diatom taxon and characterize how these interactions impact microalgal growth and lipid accumulation. Our goal was to identify species of bacteria that are beneficial and antagonistic to the growth and lipid accumulation of *Phaeodactylum tricornutum*. *P. tricornutum* was chosen as a model diatom as it is widely distributed in coastal and freshwaters and is known to accumulate lipids containing several

essential fatty acids (Desbois et al. 2009, 2010, Brennan and Owende 2010). *P. tricornutum* is one species used for biofuel production because of its high lipid content and robust growth under a variety of culture conditions (Chauton et al. 2013, Vandamme et al. 2018).

II. MATERIALS AND METHODS

2.1 Culture Preparation

A strain of *P. tricornutum*, CCMP2561, was purchased from the National Center for Marine Algae (NCMA) and maintained axenically in f/2 media. This culture (termed axenic) was co-cultured with 18 bacteria isolated from a raceway growing *P. tricornutum* at the Texas A&M AgriLife Research Mariculture Research Facility in Flour Bluff, Texas (Table 1). Identity of bacteria was the result of Lawrence Livermore National Laboratory (LLNL) molecular characterization. Two of the co-cultures contained bacteria that were unculturable/not viable and these co-cultures were not used in this study. The remaining 16 co-cultures and the axenic culture were maintained in sterile f/2 media in an incubator set to 25° C on a 12:12 hour light:dark cycle at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. All cultures were transferred to fresh sterile media approximately once per month or prior to any experiments. To confirm that cultures were not contaminated with additional bacterial strains, all cultures were plated on Marine Agar plates regularly (BD Difco™ 212185, Becton Dickinson and Company, USA). Plates were incubated for 48 hours and colonies were compared with previous isolates to ensure there was no contamination based on similarity of bacterial colony shape and color. All transfers, sample collections and culture maintenance were performed under a UV sterilized Thermo Scientific 1300 series A2 biological safety hood (Fisher Scientific, Waltham, MA, USA).

2.2 Development of Chl *a*, Cell Number, and Optical Density Relationships

To rapidly assess culture growth, proxy measurements were correlated to cell counts throughout the growth cycle. Culture cell density (c/ml), optical absorbance (OD), and pigment content (Chl-*a* concentration) were measured in three co-cultures and the axenic culture. Optical density between 650 – 750 nm is commonly used to determine microalgae growth and has a strong linear correlation with microalga biomass in mixed bacterial-microalgae cultures (Le Chavanton et al. 2013, Jia et al. 2015, Qiao et al. 2016, Lu et al. 2017). Determination of chlorophyll content is another common technique used to estimate microalgae biomass (Tchmyr et al. 2013).

All cultures were inoculated to achieve similar culture cell densities as estimated by optical absorbance readings at 655 nm. Cultures were grown for 20 days using the growing conditions listed above. All cultures were swirled and positions within the incubator randomized once per day. Samples were collected at days 0, 2, 4, 6, 8, 10, 15, and 20. A total of 15 ml was collected from each flask at each time point, with 1.5 ml used for cell counts, 13.3 ml for chlorophyll-*a* content analysis, and 200 µL for OD.

Optical density was measured using a Biotek Synergy Plate reader (BioTek, Winooski, VT, USA) at 655 nm and 750 nm wavelengths with sterile f/2 media as a blank. Chl-*a* concentration was determined after cell pellet extraction in 100% acetone with DAD detection on a HP 1100 HPLC system (Hewlett-Packard Company, Palo Alto, CA USA) using procedures described in Zimba et al. (1999). Cell counts were made using a hemocytometer. Cell count, OD_{655}, OD_{750}, and chl-*a* concentrations were co-related using the statistical package R.

2.3 Growth Experiments

All cultures were transferred to 10 ml Fisherbrand™ disposable culture tubes (Thermo Fisher Scientific, Waltham, MA, USA) with 7 ml of sterile f/2 media and grown for 20 days. Cultures were inoculated at the same cell density based on absorbance readings at 655 nm and grown using the conditions as previously described. Cultures were swirled and positions randomly changed once per day. Optical density (655 nm) was measured for each culture every other day between 9-11 AM using a Hach DR 6000 (Hach, Loveland, CO USA). This experiment was repeated on three consecutive days to determine which bacterial associations were influencing the growth of *P. tricornutum*. Growth rates were determined from cell counts using the following equation:

$$\mu = \frac{lnN_{t+1} - lnN_t}{\Delta t}$$

were N_t is the cell count at any given time, N_{t+1} is the cell count at the next sample period, and Δt is the time interval between the cell counts.

Difference in cell densities and growth rates between co-cultures and the axenic culture were determined using ANOVA at $\alpha = 0.05$, with a p-value < 0.05 indicating significance. All statistical analyses used the statistical packages R and SAS. Independent variables (experiments and cultures) were tested for interaction. Then each dependent variable was tested to see if experiment had a significant effect on the results. Data were pooled where appropriate and analyzed together. Co-cultures that exhibited consistent trends, both positive and negative, compared to the axenic culture were used for further testing based on rank.

2.4 Growth and Lipid Accumulation of Selected Strains

Six co-cultures were selected based on the growth experiments. Cell counts, growth rates, and lipid content of three co-cultures that consistently had high OD and three co-cultures that had low OD were compared to the axenic culture. All 6 co-cultures and the axenic culture were

transferred into 2 L volumes of sterile f/2 media in triplicate. All cultures were inoculated at cell densities between $3.0x10^5$ - $3.5x10^5$ (cells/ml). Cultures were grown for 6 days at 20 °C on a 12:12 hour light cycle at 275 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Each culture was mixed and its position randomized daily. Culture growth was tracked by collecting 1 ml samples and counting cells using a hemocytometer. All samples for cell number were collected between 9-11 AM on days 0, 2, 3, 4, and 6. Samples for lipid analysis were collected on day 3 and day 6 during the mid-exponential and late exponential phases of culture. On day 3 and day 6, 1 L and 900ml of each culture were collected, respectively. Samples were centrifuged in an Allegra® X-12R (Beckman Coulter Life Sciences, Indianapolis, IN, USA) at 3200*g* for 5 minutes. The supernatant was removed and pellets were collected and placed in a Stirling Ultracold -80° C freezer (Stirling Ultracold, Athens, OH, USA). The frozen cell pellets were freeze-dried using a Labconco Freezone 4.5 (Labconco, Kansas City, MO, USA). Freeze-dried samples for all co-cultures were weighed and compared to the axenic culture. Freeze-dried samples were then used for lipid analysis. Total lipid content was determined using the Folch et al. (1957) method. The freeze-dried material (100mg) was partitioned using chloroform: methanol: water separation and gravimetric assessment.

The *Marinobacter* 3-2 and *Muricauda* ARW1Y1 co-cultures were analyzed for fatty acid accumulation. The two co-cultures were grown in 2 L of sterile f/2 media in six replicates per co-culture. All cultures were inoculated at the same cell density of approximately $3.0x10^5$ as determined by OD at 655nm. Cultures were grown for 6 days at 20 °C on a 12:12 hour light cycle at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Each culture was mixed and its position randomized daily. Biomass was determined by collecting 1 ml samples every day and counting cells using a hemocytometer. All samples for cell counting were

collected between 9-10 AM. Samples for lipid analysis were collected on day 3 and day 6 during the mid-exponential and late exponential phases of growth. On day 3 half of the replicate cultures were collected and all 2 liters were harvested. The other 3 replicate cultures were harvested on day 6. Dry biomass and total lipids were determined as described above. The Bigelow et al. (2011) method was used to quantify fatty acid composition. Fatty acid analysis used 100 mg of freeze dried biomass. Fatty acid standard solution (FAME-32, SUPELCO, Bellefonte, PA, USA) was used to identify and quantify fatty acids peaks partitioned using an Agilent 6890 GC equipped with a MS 5290 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA).

Differences within and between co-cultures and the axenic culture were determined using ANOVA with significant differences having a p-value < 0.05. All statistics were run using the statistical packages R and SAS.

2.5 Bacterial Growth Inhibition Assay

Competitive inhibition between bacterial strains was assessed using methods described by Durso et al. (2004) with small variations. Three bacterial isolates, *Marinobacter* 3-2, *Algoriphagus* ARW1R1, and *Muricauda* ARW1Y1, were grown axenically in sterile Marine Broth (BD Difco^{TM} 279110, Becton Dickinson and Company, USA). All bacterial cultures were incubated at 25°C. Each bacterial culture was diluted with sterile marine broth to achieve a McFarland standard reading approximating 0.5. A 1.0ml samples from each culture was centrifuged and the resulting supernatant placed on sterile paper discs (6mm Blank Paper Discs, Becton Dickerson and Company, USA). Bacterial lawns of each culture were plated on Marine Broth agar plates. Supernatant discs were then placed onto each bacterial lawn to determine if bacterial cultures contained antimicrobial compounds. Plates were incubated for 24 hours. The

zone of growth inhibition around the discs culture were measured and the diameter of the disc subtracted. Significant differences in the size of the zone of inhibition determined using ANOVA. The assay was repeated twice.

III. RESULTS

3.1 Development of Chl *a*, Cell Number, and Optical Density Relationships

Optical density (OD) at wavelengths of 655 nm and 750 nm had the highest correlation to cell counts (Table 2). Both OD wavelengths had a Pearson coefficients (r) with cell counts of 0.96. Chlorophyll *a* content had the lowest correlation to cell count (Table 2).

The axenic culture had the highest cell count and OD (655nm & 750nm) on day 6 (Appendix 1). There was no significant difference in chl *a* content (Appendix 1). *Oceanicaulis* 6D had the lowest cell densities and OD (655nm & 750nm) on day 6 (Appendix 1). On day 20 *Oceanicaulis* 13A had the highest cell count ($4.96x10^6$ cells/ml) but did not have a significantly higher OD (655nm & 750nm) or chl-*a* content (Appendix 2).

3.2 Growth Experiments

In the combined growth experiments, the optical density (655 nm) of the axenic culture on day 6 was exceeded numerically by seven co-cultures. *Marinobacter* 3-2, *Devosia* EAB7W2, and *Algoriphagus* ARW1R1 had 16.67% higher OD on day 6 compared to the axenic culture (Table 3). *Loktanella* 4BL, *Labrenzia* 13CL, *Stappia* ARW1T, and *Alcaligenaceae* EA3 had 8.33% higher OD on day 6 compared to the axenic culture (Table 3). Two co-cultures had lower OD on day 6 compared to the axenic culture, *Marinobacter* 19DW (-16.67%) and *Muricauda* ARW1Y1 (-8.33%) (Table 3).

Five co-cultures had higher maximum OD (655 nm) compared to the axenic cultures in the three combined growth experiments (Table 3): *Devosia* EAB7W2 (14.29% higher),

Algoriphagus ARW1R1 and *Oceanicaulis* 4D (both 9.52% higher), and *Marinobacter* 3-2 and *Alcaligenaceae* EA3 (4.76% higher). Maximum OD of *Muricauda* ARW1Y1 was 9.52% lower than the axenic culture. Five of the co-cultures (*Arenibacter* ARW7G5Y1, *Alcanivorax* EA2, *Marinobacter* 19DW, *Rhodobacteria* 6CLA, and *Stappia* ARW1T) had maximum OD 4.76% lower than the axenic culture (Table 3). The remaining culture had the same maximum OD as the axenic culture (Table 3).

In the combined growth experiments, the maximum growth rate (μ_{max}) for the axenic culture was exceeded by nine co-cultures (Table 3). *Marinobacter* 3-2, *Algoriphagus* ARW1R1, and *Alcaligenaceae* EA3 had maximum growth rates 10.64% higher than the axenic culture. Two of the co-cultures had lower maximum growth rates in the three combined experiments, *Alcanivorax* EA2 (-4.26%) and *Marinobacter* 19DW (-19.15%) (Table 3).

The axenic cultures had a lower exponential growth rate than 11 of the co-cultures for the three combined growth experiments. *Marinobacter* 3-2, *Algoriphagus* ARW1R1, and *Alcaligenaceae* EA3 had the highest exponential growth rates which exceeded the axenic cultures by 12.12% (Table 3). Four co-cultures had exponential growth rates that were lower than the axenic culture by 3%, *Marinobacter* 19DW, *Muricauda* ARW1Y1, *Loktanella* 4BL, and *Arenibacter* ARW7G5Y1 (Table 3).

Two way ANOVA results identified no significant statistical interaction between the two independent variables (culture x experiment number) for any of the dependent variables (maximum OD, OD on day 6, maximum growth rate, and early exponential growth rate). Data from the three experiments was pooled for the four dependent variables: optical density on day 6, maximum optical density, exponential growth rate, and maximum growth rate (Table 4). Experiment number had a significant effect on both maximum and exponential growth rates and

these variables were not used to determine which co-cultures would be used for further testing (Table 4).

Each culture's maximum OD and OD on day 6 was ranked from highest to lowest for all three experiments was used to determine which co-cultures would undergo further testing. *Devosia* EAB7W2 had the highest mean ranking for maximum optical density (2.00) and optical density on day 6 (2.33) (Table 5 & 6). *Muricauda* ARW1Y1 had the lowest mean ranking for maximum optical density (13.33) and a ranking of 14.00 for optical density on day 6 (Table 5 & 6). *Marinobacter* 19DW had mean ranking for maximum optical density 10.00 and the lowest mean ranking for optical density on day 6 (15.00) (Table 5 & 6). Other co-cultures with notable rankings include *Marinobacter* 3-2 which had a mean ranking for maximum optical density of 4.33 and 4.67 for optical density on day 6 (Table 5 & 6). *Algoriphagus* ARW1R1 had a mean ranking for maximum optical density of 5.00 and 5.17 for optical density on day 6 (Table 5 & 6). *Alcanivorax* EA2 had mean ranking for maximum optical density of 13.33 and 11.00 for optical density on day 6 (Table 5 & 6).

3.3 Growth and Lipid Accumulation of Selected Strains

Growth curves for the 6 co-cultures and the axenic culture are shown in Figure 1. *Marinobacter* 3-2 had a significantly higher cell density when compared to the axenic culture on day 6 (Figure 2). Cell densities for *Marinobacter* 3-2 were 6.5% higher than the axenic cultures on day 6 (Table 7). *Muricauda* ARW1Y1 and *Algoriphagus* ARW1R1 had significantly lower cell densities on day 3 and day 6 compared to the axenic culture (Figure 2). Cell densities of *Muricauda* ARW1Y1 were 17% and 14% lower than the axenic cultures on day 3 and day 6, respectively (Table 6). The cell densities for *Algoriphagus* ARW1R1 were approximately 11% lower than the axenic culture on both day 3 and day 6 (Table 7). There was an average increase

of ~30% in cell densities between day 3 and day 6 for all cultures. When grown at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the second lipid experiment there was no significant difference in the cell densities of *Marinobacter* 3-2 and *Muricauda* ARW1Y1 at day 3 or day 6 (Figure 3). Again, there was an average increase of ~30% in cell densities between day 3 and 6 for *Marinobacter* 3-2 and *Muricauda* ARW1Y1.

Maximum growth rate for all cultures occurred between day 2 and day 3 (Figure 1). No co-culture had a significantly higher maximum growth rate than the axenic culture. *Muricauda* ARW1Y1 had a significantly lower maximum growth rate compared to the axenic culture (Figure 4). When grown at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the second lipid experiment there was no significant difference in the maximum growth rate between *Marinobacter* 3-2 and *Muricauda* ARW1Y1 (Figure 5).

There was no significant difference between any of the cultures in accumulated dry biomass at either day 3 or day 6 (Figure 6). There was an average increase of ~48% in accumulated dry biomass between day 3 and day 6 for all cultures. There was no significant difference in the accumulated dry biomass of *Marinobacter* 3-2 and *Muricauda* ARW1Y1 at day 3 or day 6 (Figure 7) in the second lipid experiment, when grown at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. An average increase of ~40% in accumulated dry biomass occurred between day 3 and 6 for *Marinobacter* 3-2 and *Muricauda* ARW1Y1.

Marinobacter 3-2 had significantly more total lipids per liter of culture than the axenic culture on both day 3 (54%) and day 6 (17%) (Figure 8, Table 7). *Muricauda* ARW1Y1 had significantly less total lipids per liter of culture than the axenic culture on both day 3 and day 6 by 22% and 14%, respectively (Figure 8, Table 7). *Algoriphagus* ARW1R1 had significantly less total lipids on day 6 when compared to the axenic culture by 12.5% (Figure 8, Table 7). There

was an average increase of ~246% in total lipids for all cultures between day 3 and day 6, with the exception of *Marinobacter* 3-2, which had an increase of ~158%. *Marinobacter* 3-2 grown at an irradiance of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the second lipid experiment, had significantly more accumulated total lipids compared to *Muricauda* ARW1Y1 on both day 3 and day 6 (Figure 9). In the first lipid experiment, *Marinobacter* 3-2 had 97% greater total lipid content on day 3 and 36% higher total lipid content on day 6 compared to *Muricauda* ARW1Y1. In the second lipid experiment the difference was reduced to 59% and 22% for days 3 and 6, respectively. The amount of total lipids for *Marinobacter* 3-2 increased by ~120% between days 3 and day 6 and ~180% for *Muricauda* ARW1Y1 (Figure 9).

On a per cell basis, *P. tricornutum* cells associated with *Marinobacter* 3-2 accumulated significantly more lipids than axenic *P. tricornutum* cells on both days 3 and 6 (Figure 10). On day 3 *Marinobacter* 3-2 co-cultures contained 43% more lipids than axenic cells and 11% more lipids on day 6 (Table 7). There was an average increase of ~163% in lipids per cell for all cultures between days 3 and 6 except for *Marinobacter* 3-2 which had an increase of ~98%. In the second lipid experiment using an irradiance of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, *Marinobacter* 3-2 had a significantly higher amount of lipids per cell compared to *Muricauda* ARW1Y1 on both days 3 and 6 (Figure 11). In the first lipid experiment *Marinobacter* 3-2 had 51% more lipid content per cell on day 3 and 9% more total lipid content on day 6 compared to *Muricauda* ARW1Y1. In the second lipid experiment the difference was increased to 70% and 22% for days 3 and 6, respectively. The amount of lipids per cell for *Marinobacter* 3-2 increased by ~65% between day 3 and day 6 and ~120% for *Muricauda* ARW1Y1 (Figure 11).

The total fatty acid content of *Muricauda* ARW1Y1 on day 3 was significantly higher than *Marinobacter* 3-2 (Table 8). *Muricauda* ARW1Y1 also had a significantly higher amount of

PUFA compared to *Marinobacter* 3-2 on day 3 (Table 8). *Marinobacter* 3-2 on day 3 did not contain an appreciable level of eicosadienoic acid (C20:2 n-6) or dihomo-gamma-linolenic acid (DGLA) (C20:3 n-6) (Table 10). There were no significant difference in the fatty acid profiles of *Marinobacter* 3-2 and *Muricauda* ARW1Y1 on day 6. Total fatty acid content for *Marinobacter* 3-2 increased by 29% from day 3 to day 6 (Table 9). *Marinobacter* 3-2 had an increase of ~50% for both MUFA and PUFA between day 3 and day 6 (Table 9). There was very little change in the fatty acid profiles of *Muricauda* ARW1Y1 between day 3 and day 6 (Table 9). There was no significant difference in the EPA/DHA ratio between the two cultures or between day 3 and day 6 (Table 8 & 9).

3.4 Bacterial Competitive Inhibition Assay

There was no observable competitive inhibition between any of the bacterial strains after 48 hours of incubation for both assays (Figure 12).

IV. DISCUSSION

4.1 *Marinobacter* 3-2

Several significant difference were seen in *Phaeodactylum tricornutum* co-cultured with *Marinobacter* 3-2 compared to axenic cultures. The increase in the lipid content per cell accounted for ~80% of the increase in total lipid content compared to the axenic culture. Increased growth rate and cell densities accounted for the remaining ~20% increase in total lipid content. The differences in total lipid content and lipid per cell between the axenic culture and *Marinobacter* 3-2 co-culture were more pronounced on day 3 than on day 6 (Table 4). This suggests that *Marinobacter* 3-2 does not increase lipid storage capacity of *P. tricornutum* but rather promotes the accumulation of lipids earlier in the growth cycle. The lipid content of *P. tricornutum*, as with most microalgae, is known to increase under nitrogen limitation or

increasing culture age (Liang et al. 2006, Wang et al. 2014). In the present study, *P. tricornutum* cells co-cultured with *Marinobacter* 3-2 produced significantly more lipids than the axenic cells in the first three days of growth, well before the cultures entered stationary growth phase or nutrients were limiting. In the second growth experiment *Marinobacter* 3-2 had a similar amount of total lipids and lipids per cell on day 3 as in the first experiment. Intuitively the increase of lipid accumulation earlier in the growth cycle would be ideal for improving the efficiency of algal biomass production. Current methods have used partial harvest and restock techniques, and increases in lipid accumulation early in the growth cycle would allow for more effective semi-continuous harvesting techniques.

Increases in lipid accumulation have been observed for other microalgae species when co-cultured with bacterial strains (de-Bashan et al. 2002, Leyva et al. 2014), but not for microalgae associated with bacteria from the *Marinobacter* genus. The observed enhancement in total lipids of approximately 50% compared to axenic cultures is similar to the results by Leyva et al. (2014) showing an increase of 40-60% in total lipids for *Chlorella vulgaris* when co-cultured with *Azospirillum brasilense* at 27°C. Their study found that temperature was an important factor affecting *Azospirillum brasilense-Chlorella vulgaris* co-culture. Future studies should assess if temperature changes alter the interaction between *P. tricornutum* and *Marinobacter* 3-2.

We were unable to compare the fatty acid profile of *Marinobacter* 3-2 co-cultures to the axenic culture due to issues with contamination. However, *Marinobacter* 3-2 had a very similar fatty acid profile to that of *Muricauda* ARW1Y1. The only inconsistency between the two cultures was the absence of two PUFAs on day 3, eicosadienoic acid and DGLA (Table 8). Both of these FA are elongated forms of linolenic and gamma linolenic acids (GLA) and were present

on day 6 in similar quantities as in *Muricauda* ARW1Y1. Due to the presence of EPA and DHA it appears that these cultures were elongating alpha-linolenic acid (ALA) chains, but not linoleic acid or GLA until after day 3.

Our study found that *P. tricornutum* co-cultured with *Marinobacter* 3-2 had significantly higher cell culture densities during late exponential phase of growth. These results are consistent with the findings of Amin et al. (2009) that strains of *Marinobacter* had a positive influence on microalgal growth. Amin et al. (2009) documented that increased cell culture densities of *P. tricornutum* resulted from bacterial production of vibrioferrin. Vibrioferrin binds with iron and makes it photolabile, reducing Fe (III) to Fe (II) and making it bioavailable for microalgal cells (Amin et al. 2009). Increased iron availability can increase *P. tricornutum* growth rates resulting in high cell densities (Zhao et al. 2018). Additionally, some strains of *Marinobacter* are believed to produce vitamin B_{12} (cobalamin) (Baker et al. 2016). Vitamin B_{12} promotes higher cell density in *P. tricornutum* cultures as the presence of the methionine synthase (METH) gene requires cobalamin as a cofactor for the METH enzyme (Helliwell et al. 2011). While there was a significant increase in culture cell density during the late exponential phase of growth it did not result in a significant difference in dry biomass compared to the axenic *P. tricornutum* culture.

4.2 *Muricauda* ARW1Y1

The reduced accumulation of total lipids associated with *Muricauda* ARW1Y1 co-cultures compared to the axenic cultures appears to be related to reduced cell densities. The percent difference between the axenic cultures and *Muricauda* ARW1Y1 co-cultures for total lipids and cell densities were approximately equal (Table 7). Additionally, there was no significant difference in lipid per cell between the axenic cultures and *Muricauda* ARW1Y1 co-

cultures (Table 7). These finding suggest that the lower amount of total lipids are driven by the reduction in the maximum growth rate and the subsequent lower cell densities.

Muricauda spp. have been shown to have species-specific interaction with microalgae. Le Chavanton et al. (2013) showed that a strain of *Muricauda* reduced the maximum growth rate of *Dunaliella* cultures, but increased the total biomass accumulation. In their study the maximum growth rate of the co-culture was reduced by 22% compared to the axenic strain. In the present study, *Muricauda* ARW1Y1 reduced the maximum growth rate by 13% compared to the axenic cultures. Similarly Han et al. (2016) found that two different strains of *Muricauda* had negative influences on growth rates and cell densities of *Nannochloropsis gaitana*. Han et al. (2016) reported that *N. gaitana* cells began rupturing after 5 days of co-culture, whereas the same strains had positive impacts on growth rates and cell densities of *Tetraselmis chuii* and *Cylindrotheca fusiformis* (Han et al. 2016). Shi et al. (2013) found that *Muricauda* strains can degrade *Skeletonema costatum* cells through direct contact and attachment. The inhibitory and algicidal activity observed by Han et al. (2016) and Shi et al. (2013) were found to be concentration dependent. Both studies found that higher bacterial concentrations increased the algicidal activities of the respective *Muricauda* strains (Shi et al. 2013, Han et al. 2016). In the present study, we did not track the concentration of the bacteria within the co-culture. Future work should determine how *Muricauda* ARW1Y1 concentrations impact *P. tricornutum* growth and lipid accumulation.

4.3 *Algoriphagus* ARW1R1

The reduced lipid accumulation on day 6 of *Algoriphagus* ARW1R1 co-cultures relative to the axenic cultures appears to be related to the lower culture cell densities. Total lipid content on day 6 was ~12% lower than that of the axenic cultures and the cell densities for *Algoriphagus*

ARW1R1 on day 6 were ~12.5% lower than the axenic cultures (Table 7). These results agree with the finding that there was no significant difference between the lipid per cell in *Algoriphagus* ARW1R1 and the axenic cultures. In contrast to the findings for *Muricauda* ARW1Y1, there was no significant difference between the maximum growth rate of *Algoriphagus* ARW1R1 and that of the axenic cultures. Instead *Algoriphagus* ARW1R1 had a slightly reduced growth rate throughout the entire growth cycle (Figure 1).

There is little literature on the *Algoriphagus* genus and their interactions with microalgae. One study found that a species of *Algoriphagus* had algicidal activity on several dinoflagellates species causing loss of motility and lysis within 6 hours (Manest et al. 2013). This is the only study which demonstrates algicidal occurrence by an *Algoriphagus* spp. Our work is the first to show a species of *Algoriphagus* to inhibit growth of a diatom (*P. tricornutum*). Future work should be done to see if members of the genus *Algoriphagus* inhibit the growth of other diatom species.

4.4 Other Co-cultures

No other co-culture exhibited any significant difference in growth, accumulated dry biomass, or lipid accumulation compared to the axenic culture. This result was unexpected as some of these co-cultures had significant differences in growth compared to the axenic cultures in at smaller culture volumes (Appendix 3-5). It has been noted that microalgal cultures may behave differently at differing culture volumes (Zhang et al. 2018). *P. tricornutum* also had significantly different lipid accumulation based on the species/strain of *Marinobacter* (Figures 8-9). This supports the idea that bacterial-microalgal interaction are highly specific to the species of bacteria and microalgae (Le Chavanton et al. 2013, Kim et al. 2014, Wang et al. 2016).

4.5 Culture Performance

Phaeodactylum tricornutum has been used as a model diatom in physiology and growth studies for decades and consequently there are many papers reporting growth rates and lipid content from strains under varying culture conditions. Additionally, many studies involving *P. tricornutum* have not used axenic cultures and bacterial associations are unknown. Different strains of *P. tricornutum* have been shown to have different optimal growing conditions, growth rates, lipid accumulation, and fatty acid profiles (Siron et al 1989, Yongmanitchai et al. 1991, De Martino et al. 2007, Qiao et al. 2016, Steinrucken et al. 2018, Vandamme et al. 2018). Likewise the impact of phycosphere-associated bacteria on microalgae cultures is known to vary with culture conditions (Le Chavanton et al. 2013, Shi et al. 2013, Leyva et al. 2014, and Han et al. 2016). These factors make it difficult to compare the results of this study to those in the literature.

Lipid accumulation of between 11-15% dry weight in *P. tricornutum* CCMP 2561 during the present study is consistent with the 10% dry weight reported in Chauton et al. (2013) (Table 9). According to Vandamme et al. (2018) this strain can accumulate much higher lipid content, reaching 35% dry weight after 13 days of culture. Variations in the growth media and aeration methods could explain the lower lipid accumulation observed in this study.

The total fatty acid content (TFAC) of our cultures was consistent with the finding of Yongmanitchai et al. 1991 of 18-31% dry weight; however, TFAC values of *P. tricornutum* were highly variable (Table 10). The present study showed an increase in the ratio of unsaturated/saturated fatty acids for *Marinobacter* 3-2 co-cultures from day 3 to 6. This is consistent with the finding of Siron et al. (1989) who reported a similar trend from exponential to late exponential phase of growth. A similar ratio change was not found in *Muricauda* ARW1Y1 co-cultures. Qiao et al. (2016) reported no change in the ratio of unsaturated to saturated fatty

acids for *P. tricornutum*, however their cultures were no longer in the exponential phase of growth when samples were collected. Similarly, Siron et al. (1989) suggests that ratios of unsaturated:saturated fatty acids are relatively stable during the linear phase of growth. The stable fatty acid profile of the *Muricauda* ARW1Y1 co-cultures was suprising as cultures were still in exponential phase and not stationary.

The concentration of EPA in algal co-cultures in the present study was low compared to published studies (Table 11). Conversely, the DHA content was higher (Table 11). As a result, the EPA/DHA ratio in our study (1.19-1.20) is much lower than previously reported (2-35) (Siron et al. 1989, Veloso et al. 1991, Yongmanitchai et al. 1992, Qiao et al. 2016, Boelen et al. 2017). The EPA/DHA ratio of *P. tricornutum* cultures seem to be highly variable. The EPA/DHA ratio in co-cultures of algae remained relatively constant between day 3 and day 6 changing less than 1%. Boelen et al. (2017) found that the EPA/DHA ratio of *P. tricornutum* decreases only slightly between the exponential phase and the beginning of the stationary phase. In the present study the content of EPA and DHA were approximately equal and their ratios remained stable throughout the growth cycle.

The maximum growth rates observed in the present study were lower than the findings of De Martino et al. (2007) for the axenic strain CCMP 2561. De Martino reported a maximum growth instantaneous growth rate (μ) of 0.97 vs. 0.61 – 0.77 in the present study. In fact, maximum growth rates were lower than those reported for most strains of *P. tricornutum* (Table 12). Culture conditions in all of these studies varied; however, all cultures were grown at temperatures between 18° and 20° C. Qiao et al. (2016) showed a significant difference in growth rate based on the growth temperature with an optimal temperature of 20° C for their strain. Similarly we observed higher growth rates in our cultures when grown at 20° C compared

to 25°C (Figure 3, Appendix 3-5). The most notable difference between growth conditions in the present study and other comparable studies was the level of irradiance. De Martino et al. (2007) used between 60 – 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, Siron et al. (1989) 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$, Li et al. (2012) 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and Li et al. (2014) 460 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with little effect on growth rates. In the second lipid experiment, an irradiance of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was used and we observed similar growth rates over the first three days compared to the cultures grown at 275 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After day 3, the cultures grown at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had reduced growth rates compared to the cultures with higher levels of irradiance. It is likely that at high cell densities, 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was not sufficient to support maximum growth as light becomes more limited in denser cultures. Tolerance to high levels of irradiance could be a trait specific to certain strains of *P. tricornutum*. Due to its relevance for genomic studies, optimal growth conditions for *P. tricornutum* strain CCMP 2561 should be determined in axenic and co-culture.

V. CONCLUSION

Bacterial phycosphere-association can have a wide range of effects on microalgae growth and lipid accumulation. A specific strain of *Marinobacter* (3-2) increased the total amount of lipid accumulation of *P. tricornutum* significantly compared to axenic cultures. These attributes plus the increase in cell densities would be beneficial to the commercial culturing of *P. tricornutum* for biofuel production. Future work should determine how culture condition impacts this bacterial algal interaction and how *Marinobacter* 3-2 impacts the growth and physiology of other lipid-rich microalgae species.

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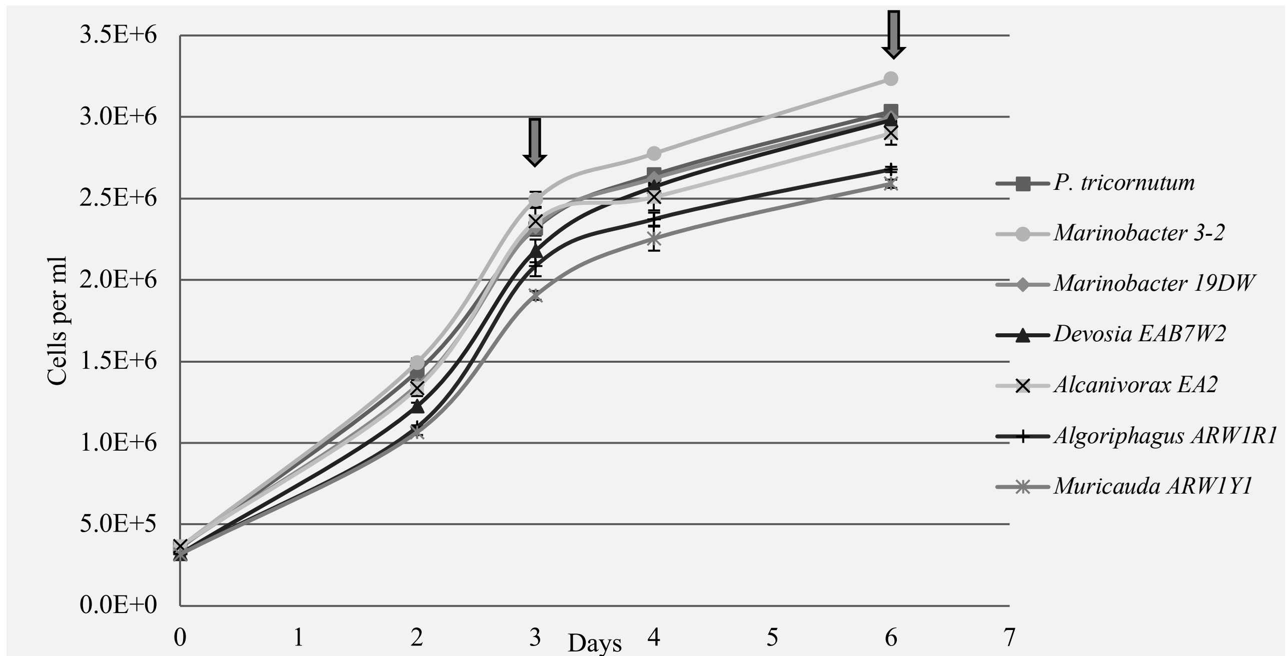

Figure 1: Growth curves of *P. tricornutum* co-cultures and the axenic culture in the lipid experiment. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 275 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Each point is the mean of triplicate samples. Error bars are the standard deviation. Arrows show when samples were collected for lipid analysis.

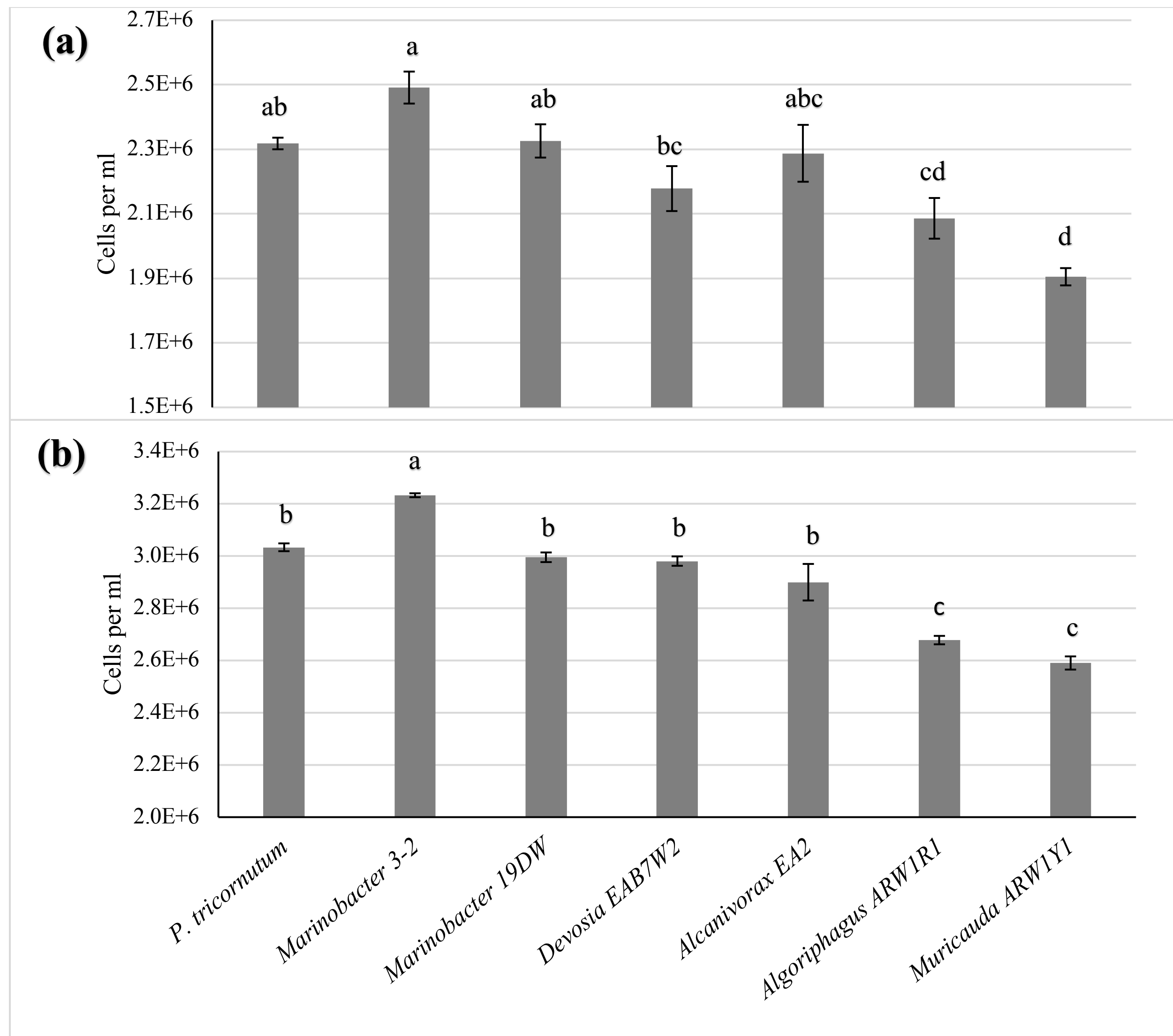

Figure 2: Cell counts per milliliter of *P. tricornutum* co-cultures and the axenic culture in the lipid experiment. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 275 µmol·m^{-2}·s^{-1} PAR. (a) Cell counts on day 3. (b) Cell counts on day 6. Bars indicate the means and error bars are the standard deviations. Cultures with the same letters are not significantly different from each other.

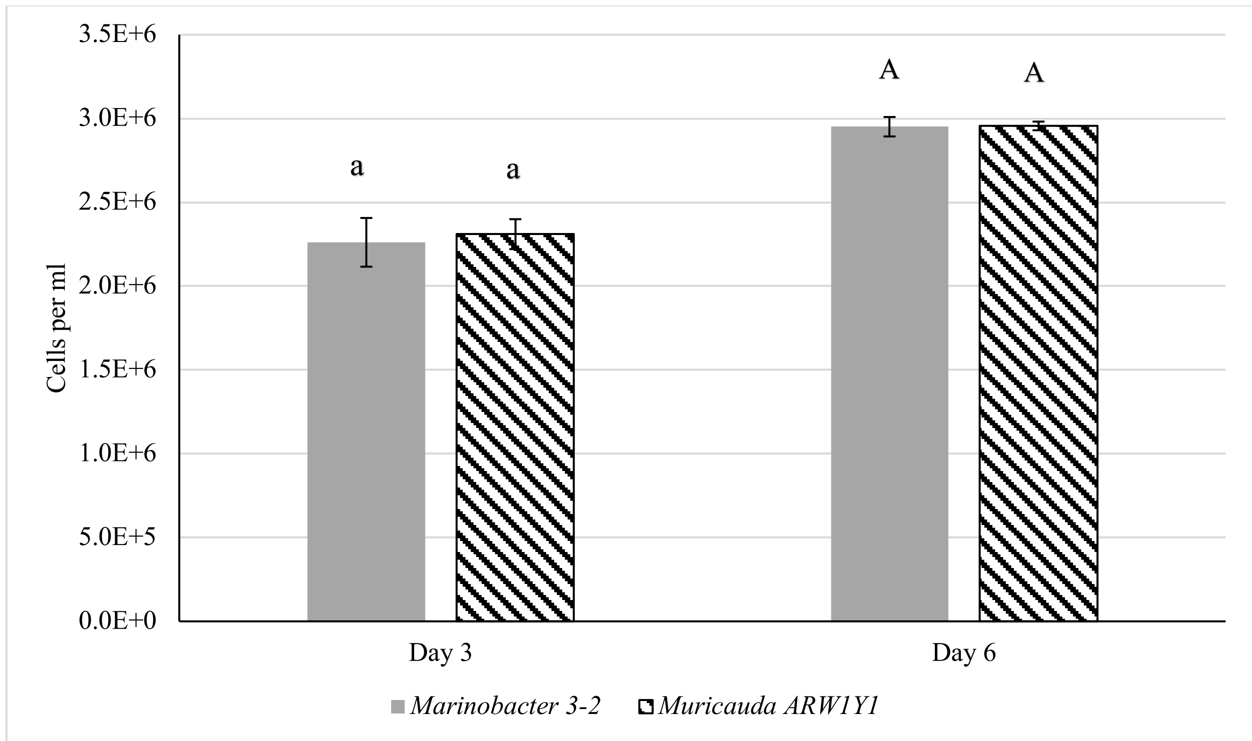

Figure 3: Cell counts per milliliter of *Marinobacter* 3-2 and *Muricauda* ARW1Y1 co-cultures. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Bars indicate the means and error bars are the standard deviations. Cultures with the same letters are not significantly different from each other. Lower case letters compare cell densities on day 3 and capital letters compare cell densities for day 6.

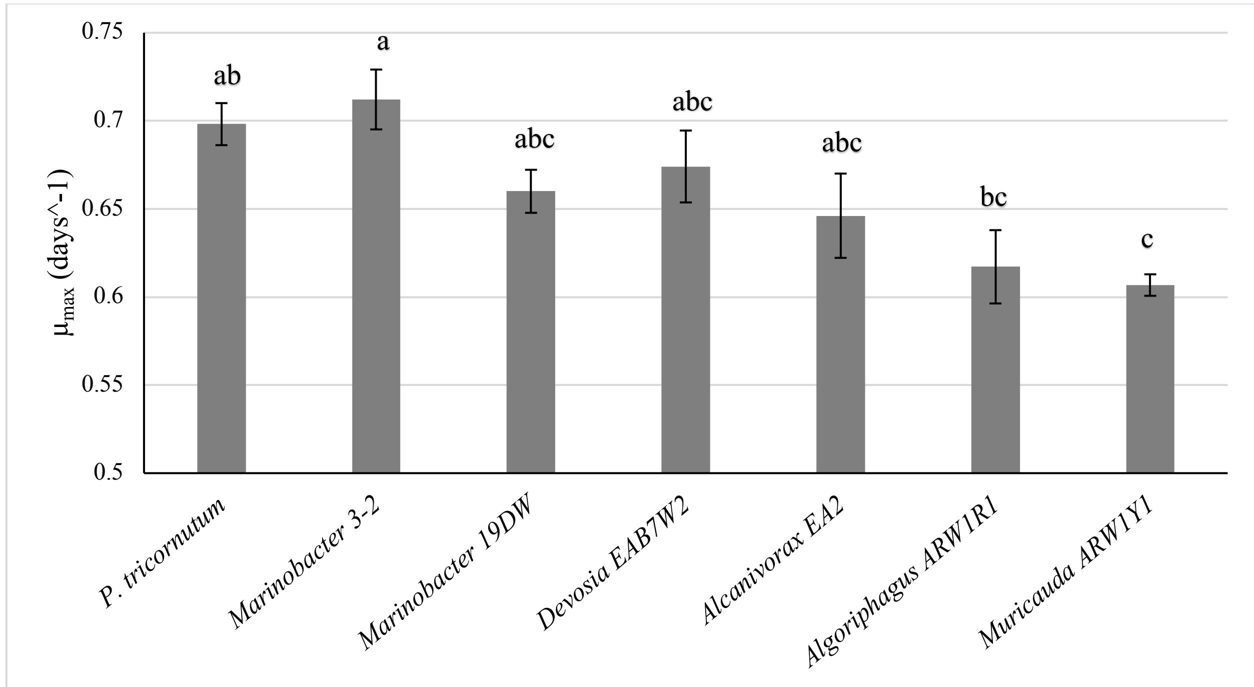

Figure 4: Maximum growth rate (μ_{max}) of *P. tricornutum* co-cultures and the axenic culture in the lipid experiment. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 275 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Maximum growth occurred on day 2. Growth rates determined using cell counts. Bars indicate the means and error bars are the standard deviations. Cultures with the same letters are not significantly different from each other.

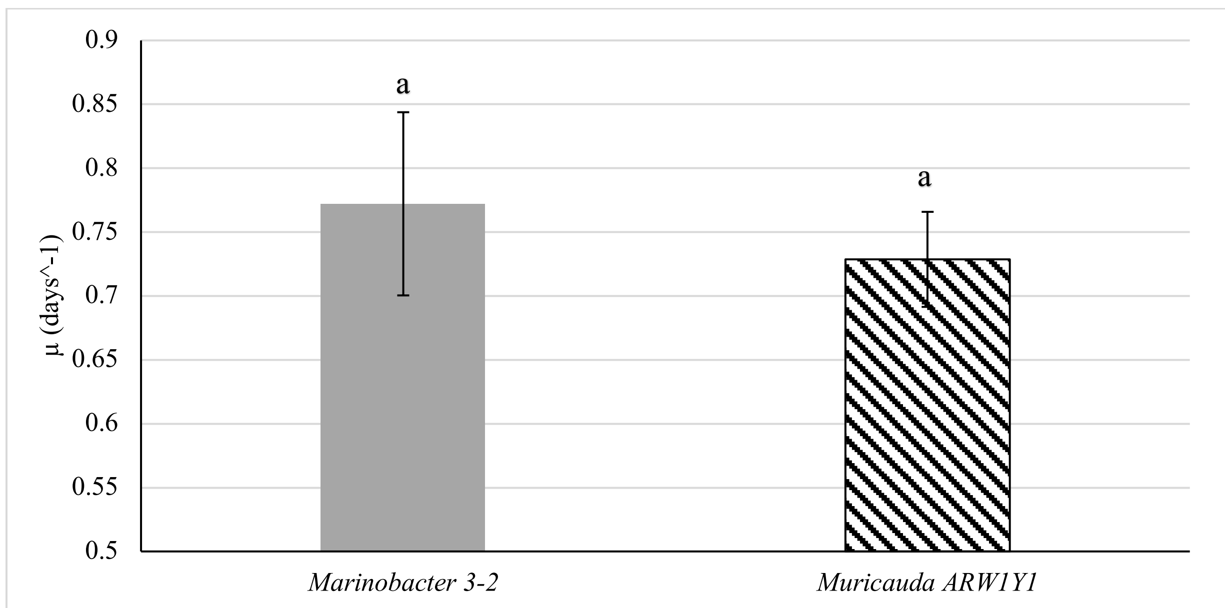

Figure 5: Maximum growth rate (μ_{max}) of *Marinobacter* 3-2 and *Muricauda* ARW1Y1 co-cultures. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Growth rates determined using cell counts. Bars indicate the means and error bars are the standard deviations. Cultures with the same letters are not significantly different from each other.

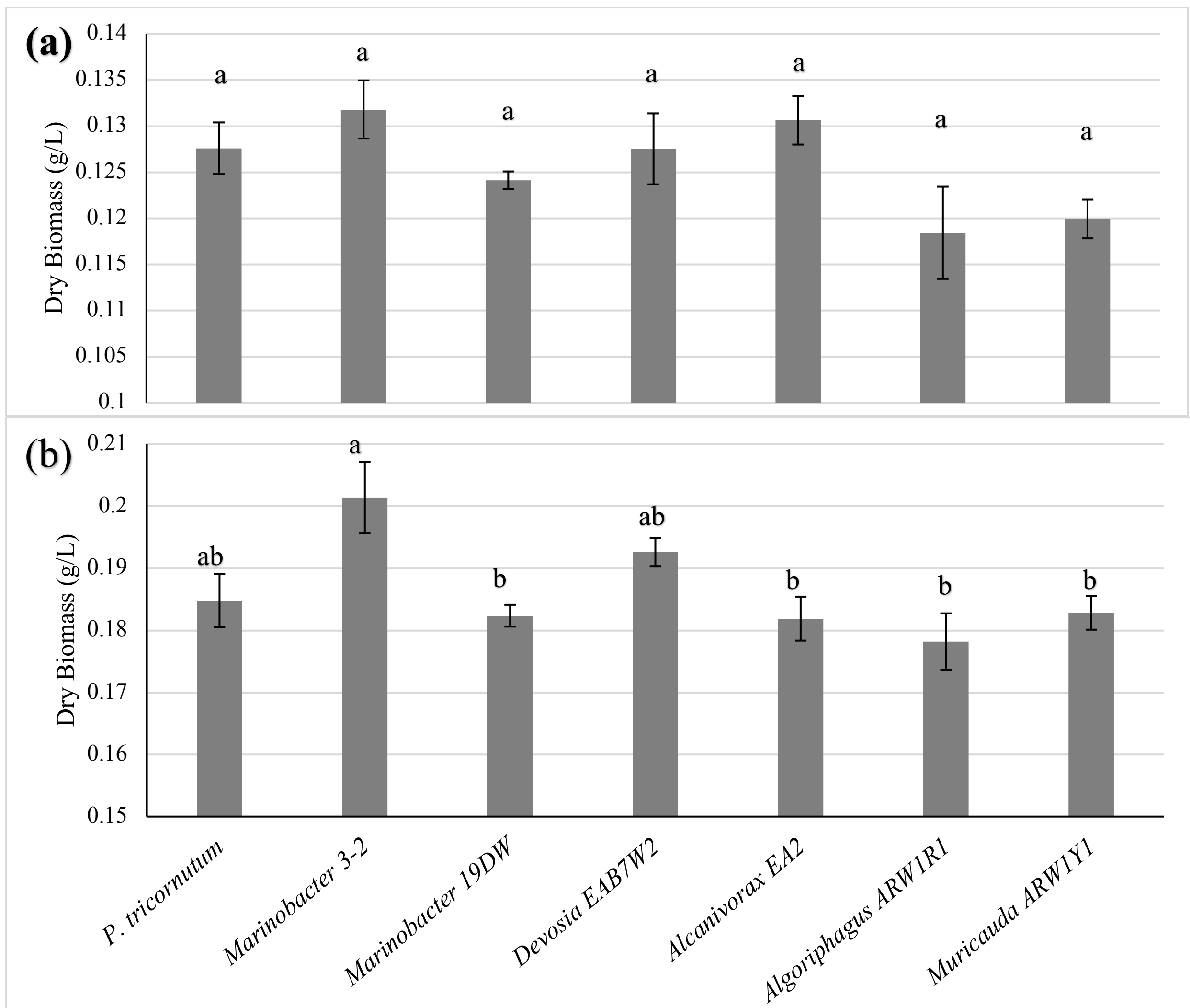

Figure 6: Accumulated dry biomass (g/L) of *P. tricornutum* co-cultures and the axenic culture in the lipid experiment. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 275 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. (a) Dry biomass collect on day 3. (b) Dry biomass collected on day 6. Bars indicate the means and error bars are the standard deviations. Cultures with the same letters are not significantly different from each other.

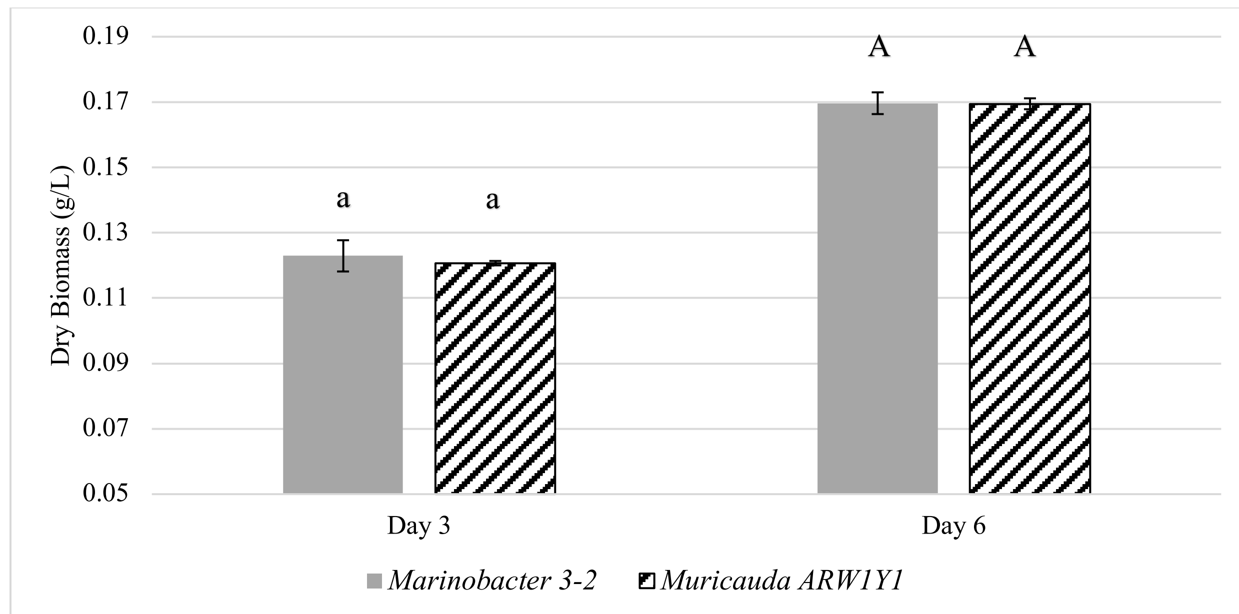

Figure 7: Accumulated dry biomass (g/L) of *Marinobacter* 3-2 and *Muricauda* ARW1Y1 co-cultures. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Bars indicate the means and error bars are the standard deviations. Cultures with the same letters are not significantly different from each other. Lower case letters compare cell densities on day 3 and capital letters compare cell densities for day 6.

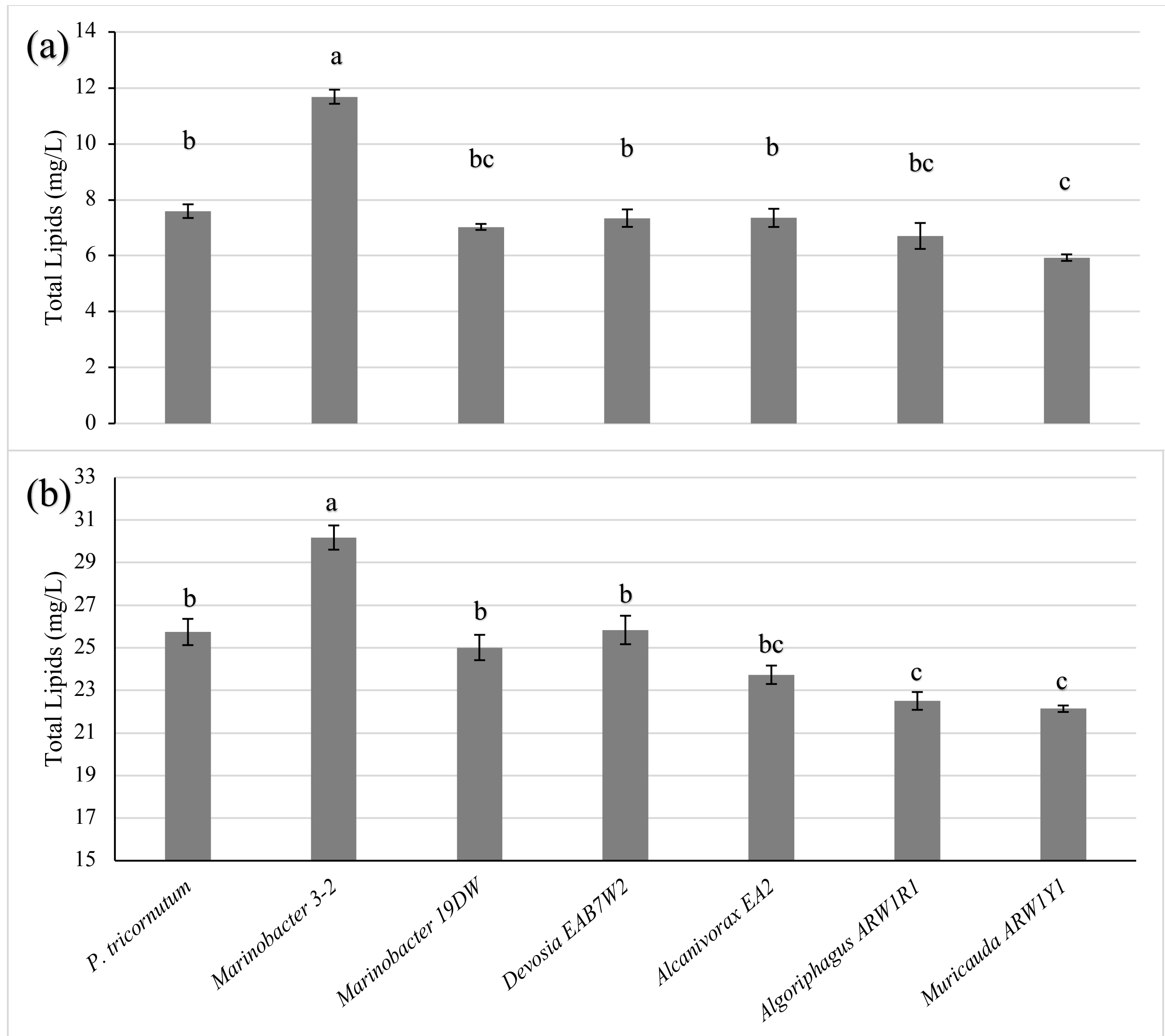

Figure 8: Total lipid content (as mg/L) of *P. tricornutum* co-cultures and the axenic culture in the lipid experiment. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 275 µmol·m^{-2}·s^{-1} PAR. (a) Total lipids on day 3. (b) Total lipids on day 6. Lipid content was determined using Folch et al. (1957). Bars indicate the means and error bars are the standard deviations. Cultures with the same letters are not significantly different from each other.

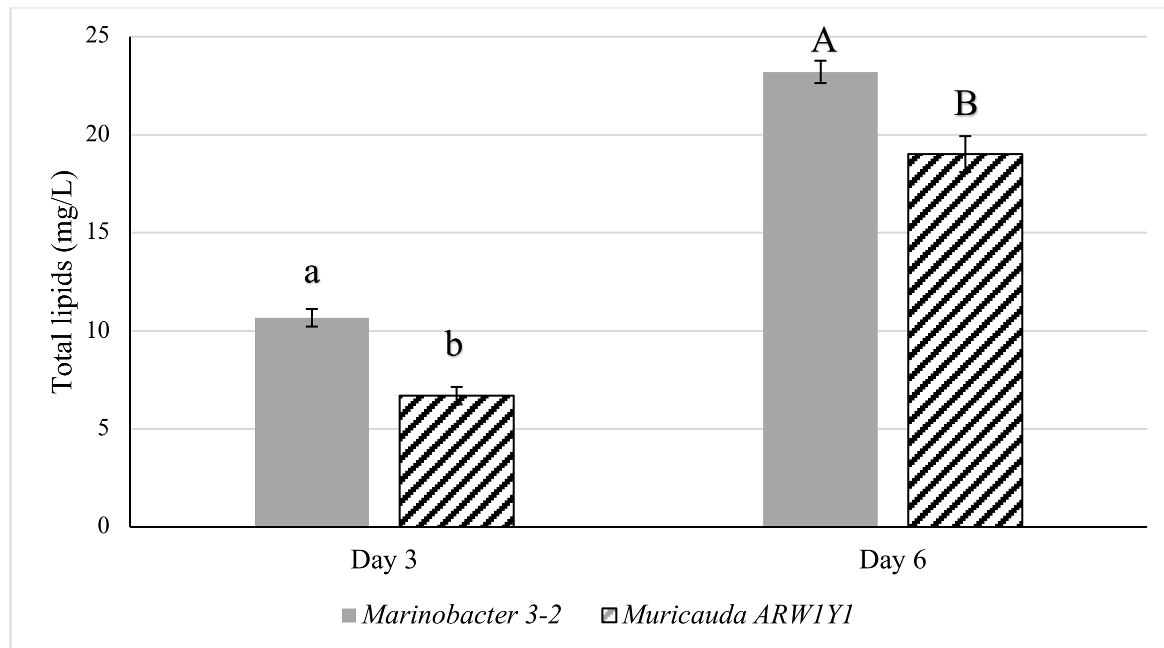

Figure 9: Total lipid content (as mg/L) of *Marinobacter* 3-2 and *Muricauda* ARW1Y1 co-cultures. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Lipid content was determined using Folch et al. (1957). Bars indicate the means and error bars are the standard deviations. Cultures with the same letters are not significantly different from each other. Lower case letters compare cell densities on day 3 and capital letters compare cell densities for day 6.

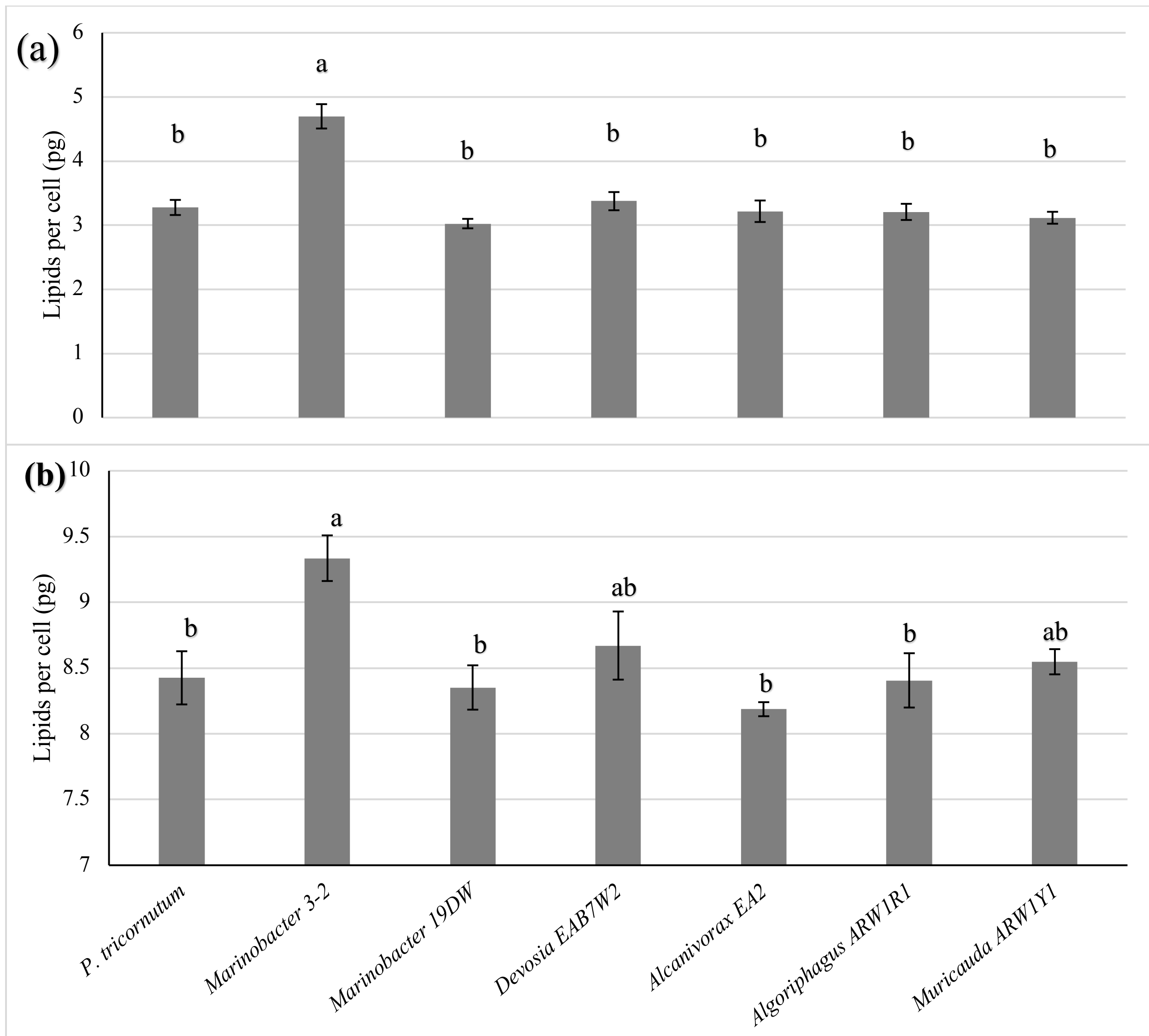

Figure 10: Lipid content (as pg/cell) of *P. tricornutum* co-cultures and the axenic culture in the lipid experiment. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 275 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. (a) Lipids per cell on day 3. (b) Lipids per cell on day 6. Lipid content was determined using Folch et al. (1957). Bars indicate the means and error bars are standard deviations. Cultures with the same letters are not significantly different from each other.

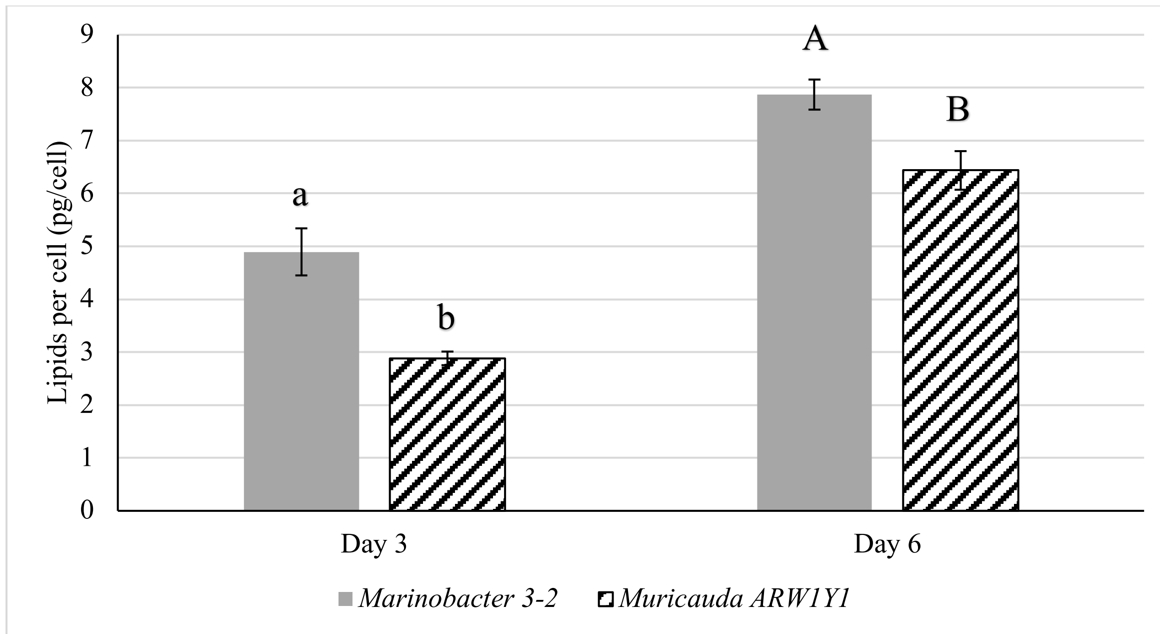

Figure 11: Lipid content (as pg/cell) of *Marinobacter* 3-2 and *Muricauda* ARW1Y1 co-cultures. Cultures where grown in f/2 media at 20°C on a 12:12 hour light:dark cycle at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Lipid content was determined using Folch et al. (1957). Bars indicate the means and error bars are the standard deviations. Cultures with the same letters are not significantly different from each other. Lower case letters compare cell densities on day 3 and capital letters compare cell densities for day 6.

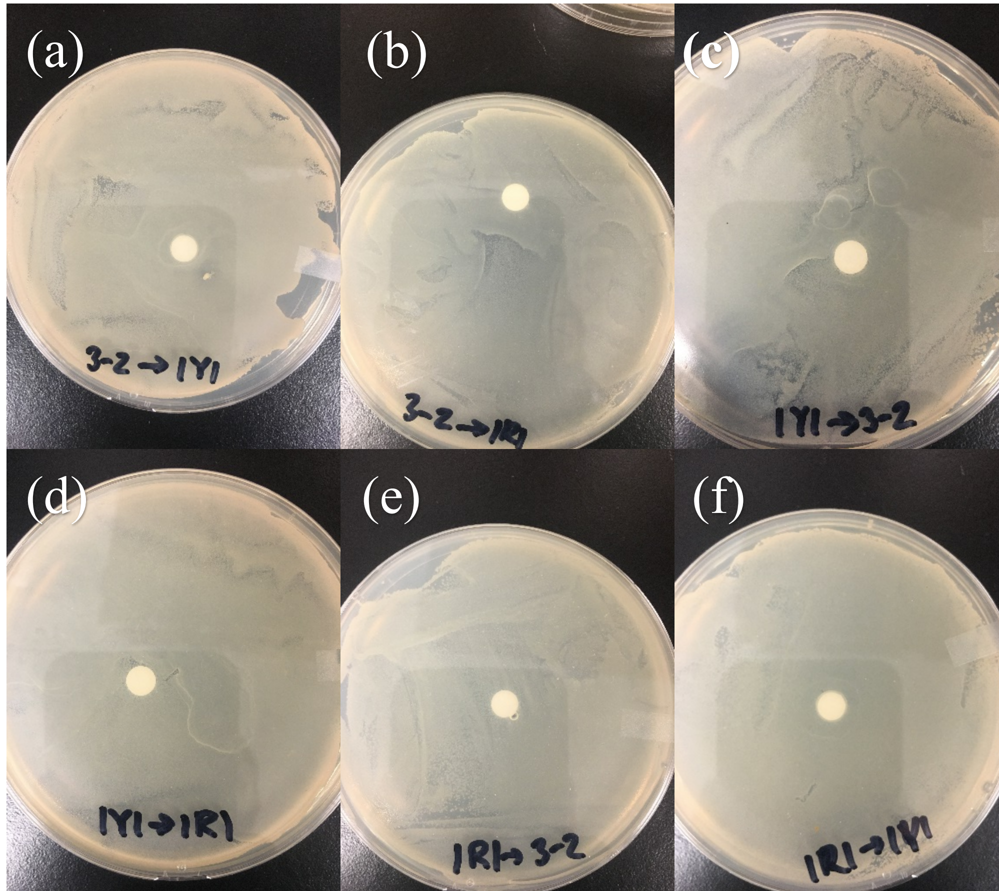

Figure 12: Inhibition assay between bacterial isolates from *P. tricornutum* co-cultures. Bacterial cultures were diluted to McFarland standard of 0.5 and steaked on marine agar plates to form a lawn. Supernatant (25 µL) from different bacterial cultures was placed on sterile paper discs and placed on the lawns. Plates were incubated at 30°C for 24 hours. (a) *Marinobacter* 3-2 bacterial lawn with *Muricauda* ARW1Y1 supernatant disc. (b) *Marinobacter* 3-2 bacterial lawn with *Algoriphagus* ARW1R1 supernatant disc. (c) *Muricauda* ARW1Y1 bacterial lawn with *Marinobacter* 3-2 supernatant disc. (d) *Muricauda* ARW1Y1 bacterial lawn with *Algoriphagus* ARW1R1 supernatant disc. (e) *Algoriphagus* ARW1R1 bacterial lawn with *Marinobacter* 3-2 supernatant disc. (f) *Algoriphagus* ARW1R1 bacterial lawn with *Muricauda* ARW1Y1 supernatant disc.

Table 1: Taxonomic identity of bacterial isolates from water samples collected at Texas A&M AgriLife Mariculture Research Facility located in Flour Bluff, Texas.

Bacterial Identification
Arenibacter ARW7G5Y1
Muricauda ARW1Y1
Muricauda ARW7G5W
Algoriphagus ARW1R1
Oceanicaulis 13A
Oceanicaulis 4D
Hyphomonadaceae 6ES
Devosia EAB7W2
Labrenzia 13C1
Loktanella 4BL
Stappia ARW1T
Rhodobacteraceae 6CLA
Thalassospira 11-3
Alphaproteobacteria EA10
Alcaligenaceae EA3
Marinobacter 3-2
*Marinobacter*19DW
Alcanivorax EA2

Table 2: Pearson correlation between cell counts, optical density, and chlorophyll *a* content of *P. tricornutum* cultures co-cultured with individual bacterial strains and an axenic *P. tricornutum* culture. Cultures where grown in f/2 media at 25°C on a 12:12 hour light:dark cycle at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR.

	Cell count	OD655	OD750	Chl-*a*
Cell count	1			
OD655	0.96	1		
OD750	0.96	0.98	1	
Chl-*a*	0.60	0.61	0.57	

Table 3: Mean and standard deviation of optical density (OD) and growth rates of *P. tricornutum* co-cultures and the axenic culture from the three consecutive growth experiments. Cultures where grown in f/2 media at 25°C on a 12:12 hour light:dark cycle at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. OD was measured at 655 nm using a Hach DR 6000 system. Cultures with the same letters are not significantly different from co-cultures in the same column.

Culture	Maximum OD	OD Day 6	Maximum growth rate (μ_{max})	Exponential growth rate (μ)
Axenic	0.21 ± 0.03^{ab}	0.12 ± 0.02^{bc}	0.47 ± 0.07^{ab}	0.33 ± 0.02^{ab}
Loktanella 4BL	0.21 ± 0.02^{bc}	0.13 ± 0.01^{ab}	0.47 ± 0.07^{ab}	0.32 ± 0.01^{b}
Oceanicaulis 4D	0.23 ± 0.01^{ab}	0.12 ± 0.01^{bc}	0.5 ± 0.07^{a}	0.35 ± 0.01^{ab}
Rhodobacteria 6CLA	0.20 ± 0.02^{c}	0.12 ± 0.01^{bc}	0.47 ± 0.03^{ab}	0.34 ± 0.01^{ab}
Oceanicaulis 13A	0.21 ± 0.01^{ab}	0.12 ± 0.01^{bc}	0.47 ± 0.05^{ab}	0.34 ± 0.03^{ab}
Labrenzia 13C1	0.21 ± 0.02^{ab}	0.13 ± 0.01^{abc}	0.47 ± 0.05^{ab}	0.35 ± 0.02^{ab}
Marinobacter 19DW	0.20 ± 0.02^{bc}	0.10 ± 0.02^{d}	0.38 ± 0.09^{b}	0.32 ± 0.05^{b}
Devosia EAB7W2	0.24 ± 0.01^{a}	0.14 ± 0.01^{a}	0.51 ± 0.04^{a}	0.36 ± 0.03^{ab}
Stappia ARW1T	0.20 ± 0.02^{bc}	0.13 ± 0.01^{abc}	0.49 ± 0.03^{a}	0.34 ± 0.01^{ab}
Algoriphagus ARW1R1	0.23 ± 0.02^{a}	0.14 ± 0.01^{ab}	0.52 ± 0.04^{a}	0.37 ± 0.02^{a}
Muricauda ARW1Y1	0.19 ± 0.01^{c}	0.11 ± 0.01^{cd}	0.50 ± 0.05^{a}	0.32 ± 0.03^{ab}
Arenibacter ARW7G5Y1	0.20 ± 0.02^{bc}	0.12 ± 0.01^{bc}	0.49 ± 0.05^{a}	0.32 ± 0.02^{b}
Alcanivorax EA2	0.20 ± 0.01^{bc}	0.12 ± 0.01^{bc}	0.45 ± 0.06^{ab}	0.33 ± 0.02^{ab}
Alcaligenaceae EA3	0.22 ± 0.02^{ab}	0.13 ± 0.02^{ab}	0.52 ± 0.06^{a}	0.37 ± 0.03^{a}
Alphaproteobacteria EA10	0.21 ± 0.01^{ab}	0.12 ± 0.01^{bc}	0.49 ± 0.06^{a}	0.33 ± 0.04^{ab}
Marinobacter 3-2	0.22 ± 0.01^{ab}	0.14 ± 0.01^{ab}	0.52 ± 0.04^{a}	0.37 ± 0.02^{a}

Table 4: F values and P values for the three growth experiments. *P. tricornutum* cultures were grown in f/2 media at 25°C on a 12:12 hour light:dark cycle at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. OD was measured at 655 nm using a Hach DR 6000 system.

Dependent variable	Independent variable	DF	F Value	Pr > F
Maximum optical density	Culture	15	3.00	0.0006
	Experiment	2	0.15	0.8641
Optical density day 6	Culture	15	3.93	<0.0001
	Experiment	2	1.32	0.2729
Maximum growth rate	Culture	15	3.89	<0.0001
	Experiment	2	13.05	<0.0001
Exponential growth rate	Culture	15	2.66	0.0021
	Experiment	2	10.34	<0.0001

Table 5: Relative rank of the maximum optical density of *P. tricornutum* cultures in each experiment. Mean and standard deviations given for the rank of the three experiments. Cultures where grown in f/2 media at 25°C on a 12:12 hour light:dark cycle at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. OD was measured at 655 nm using a Hach DR 6000 system. Cultures and values in bold were chosen for further experimentation.

Culture	Experiment 1	Experiment 2	Experiment 3	Mean	SD
Axenic	4	14	14	10.67	5.77
Loktanella 4BL	8	16	7	10.33	4.93
Oceanicaulis 4D	3	6	3	4.00	1.73
Rhodobacteria 6CLA	16	9	11	12.00	3.61
Oceanicaulis 13A	7	13	8	9.33	3.21
Labrenzia 13C1	13	5	5	7.67	4.62
Marinobacter 19DW	14	10	6	10.00	4.00
Devosia EAB7W2	1	4	1	2.00	1.73
Stappia ARW1T	5	15	15	11.67	5.77
Algoriphagus ARW1R1	2	1	12	5.00	6.08
Muricauda ARW1Y1	12	12	16	13.33	2.31
Arenibacter ARW7G5Y1	15	8	9	10.67	3.79
Alcanivorax EA2	11	11	13	11.67	1.15
Alcaligenaceae EA3	10	3	4	5.67	3.79
Alphaproteobacteria EA10	6	7	10	7.67	2.08
Marinobacter 3-2	9	2	2	4.33	4.04

Table 6: Relative rank of the optical density on day 6 of *P. tricornutum* cultures in each experiment. Mean and standard deviations given for the rank of the three experiments. Cultures where grown in f/2 media at 25°C on a 12:12 hour light:dark cycle at 300 µmol·m^{-2}·s^{-1} PAR. OD was measured at 655 nm using a Hach DR 6000 system. Cultures and values in bold were chosen for further experimentation.

Culture	Experiment 1	Experiment 2	Experiment 3	Mean	SD
Axenic	2	12	15	9.67	6.81
Loktanella 4BL	5	13	3	7.00	5.29
Oceanicaulis 4D	7.5	7	12	8.83	2.75
Rhodobacteria 6CLA	13	6	8	9.00	3.61
Oceanicaulis 13A	6	15	11	10.67	4.51
Labrenzia 13C1	10	2	5	5.67	4.04
Marinobacter 19DW	16	16	13	15.00	1.73
Devosia EAB7W2	1	5	1	2.33	2.31
Stappia ARW1T	3	9	4	5.33	3.21
Algoriphagus ARW1R1	7.5	1	7	5.17	3.62
Muricauda ARW1Y1	12	14	16	14.00	2.00
Arenibacter ARW7G5Y1	15	4	6	8.33	5.86
Alcanivorax EA2	14	10	9	11.00	2.65
Alcaligenaceae EA3	11	2	10	7.67	4.93
Alphaproteobacteria EA10	4	8	14	8.67	5.03
Marinobacter 3-2	9	3	2	4.67	3.79

Table 7: Percent difference between *P. tricornutum* co-cultures and axenic culture for cell density, accumulated dry biomass, total lipids, and lipids per cell on day 3 and day 6. Cultures were grown in two liters of f/2 media at 20°C on a 12:12 hour light:dark cycle at 275 µmol·m^{-2}·s^{-1} PAR. Capital letters compare percent increases relative to the axenic culture and lower case letters compare percent decreases relative to the axenic culture.

	Cell Densities		Dry Biomass		Total Lipids		Lipids per Cell	
	Day 3	Day 6	Day 3	Day 6	Day 3	Day 6	Day 3	Day 6
Marinobacter 3-2	7.48^{A}	6.57^{A}	3.29^{A}	9.02^{A}	53.90^{A}	17.23^{A}	43.37^{A}	10.80^{A}
Marinobacter 19DW	0.34^{B}	-1.26^{a}	-2.72^{b}	-1.3^{a}	-7.41^{b}	-2.83^{a}	-7.68^{c}	-0.87^{a}
Devosia EAB7W2	-6.03^{b}	-1.74^{a}	-0.05^{a}	4.25^{B}	-3.29^{a}	0.37^{B}	3.00^{B}	2.91^{B}
Alcanivorax EA2	-1.31^{a}	-4.41^{b}	2.38^{A}	-1.56^{a}	-3.16^{a}	-7.81^{b}	-1.80^{a}	-2.83^{b}
Algoriphagus ARW1R1	-11.12^{c}	-11.72^{c}	-7.18^{c}	-3.57^{b}	-11.67^{bc}	-12.58^{c}	-2.11^{a}	-0.23^{a}
Muricauda ARW1Y1	-17.82^{d}	-14.61^{d}	-6.01^{c}	-1.06^{a}	-21.88^{c}	-14.00^{c}	-4.90^{b}	1.45^{B}

Table 8: Fatty acid composition (mg/g) and total fatty acid content (TFAC) of *P. tricornutum* co-cultures *Marinobacter* 3-2 and *Muricauda* ARW1Y1. Cultures were grown in two liters of f/2 media at 20°C on a 12:12 hour light:dark cycle at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Values are means of replicate cultures (mean ± standard deviation). Values within the same row with different letters are significantly different from each other. Lower case letters compare values for day 3 and capital letters compare values on day 6.

Fatty acid	Day 3		Day 6	
	Marinobacter 3-2	*Muricauda* ARW1Y1	*Marinobacter* 3-2	*Muricauda* ARW1Y1
C14.0	23.76 ± 0.46[a]	25.05 ± 1.22[a]	26.88 ± 0.72[A]	25.23 ± 0.90[A]
C15:0	9.37 ± 0.16[a]	9.84 ± 0.52[a]	10.59 ± 0.27[A]	9.96 ± 0.37[A]
C16:0	37.24 ± 0.85[a]	39.53 ± 1.97[a]	42.31 ± 1.29[A]	39.94 ± 1.38[A]
C16:1	13.92 ± 0.27[a]	14.76 ± 0.70[a]	15.84 ± 0.47[A]	14.87 ± 0.57[A]
C17:0	11.56 ± 0.23[a]	12.06 ± 0.55[a]	12.91 ± 0.43[A]	12.26 ± 0.37[A]
C18:0	17.83 ± 0.44[a]	18.98 ± 1.08[a]	20.27 ± 0.80[A]	19.17 ± 0.53[A]
C18:1	9.98 ± 0.48[a]	10.36 ± 0.47[a]	11.12 ± 0.34[A]	10.55 ± 0.33[A]
C18:2(n-6)	11.27 ± 0.15[a]	11.87 ± 0.67[a]	12.91 ± 0.37[A]	12.00 ± 0.51[A]
C18:3(n-3)	9.58 ± 0.31[a]	9.90 ± 0.70[a]	10.74 ± 0.26[A]	9.93 ± 0.47[A]
C18:3(n-6)	12.61 ± 0.25[a]	13.20 ± 0.67[a]	14.12 ± 0.43[A]	13.38 ± 0.48[A]
C20:2(n-6)		7.45 ± 0.49	11.26 ± 0.64[A]	10.97 ± 0.31[A]
C20:3(n-6)		11.02 ± 0.20	11.60 ± 0.35[A]	11.12 ± 0.16[A]
C20:5(n-3)	13.99 ± 0.29[a]	14.85 ± 0.70[a]	15.87 ± 0.46[A]	14.91 ± 0.58[A]
C22:6(n-3)	11.66 ± 0.31[a]	12.45 ± 0.50[a]	13.26 ± 0.36[A]	12.53 ± 0.43[A]
C24:0	21.90 ± 0.48[a]	23.75 ± 1.30[a]	25.17 ± 0.79[A]	23.86 ± 0.68[A]
SFA	121.66 ± 2.53[a]	129.20 ± 6.59[a]	138.12 ± 4.06[A]	130.32 ± 4.04[A]
MUFA	17.24 ± 5.90[a]	25.12 ± 1.17[a]	26.96 ± 0.78[A]	25.41 ± 0.89[A]
PUFA	59.11 ± 1.06[b]	80.74 ± 9.86[a]	89.78 ± 2.64[A]	81.19 ± 8.95[A]
EPA/DHA	1.20 ± 0.01[a]	1.19 ± 0.01[a]	1.20 ± 0.01[A]	1.19 ± 0.01[A]
TFAC	198.02 ± 8.53[b]	235.06±17.60[a]	254.85 ± 7.48[A]	236.92±13.88[A]

Table 9: Percent change in fatty acid classes and EPA/DHA ratio of *P. tricornutum* co-cultures *Marinobacter* 3-2 and *Muricauda* ARW1Y1 from day 3 to day 6. Cultures were grown in 2 liters of f/2 media at 20°C on a 12:12 hour light:dark cycle at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR.

Culture	TFAC	SFA	MUFA	PUFA	EPA/DHA
Marinobacter 3-2	28.7	13.53	56.32	51.87	-0.26
Muricauda ARW1Y1	0.79	0.87	1.16	0.56	-0.27

Table 10: Comparison of total lipid content (% DW) of *P. tricornutum* cultures between the present study and other relevant literature. Total lipid analysis for all studies determined using Folch et al. (1957).

Total lipid content (% dry weight)	*P. tricornutum* strain	Study
11-15	CCMP 2561	Present study
10-20	CCMP 2561	Chauton et al. 2013
19-25	Unidentified	Gao et al. 2017
32	Unidentified	Yang et al. 2017
9	Unidentified	Branco-Vieira et al. 2018
32-42	CCMP 2561	Vandamme et al. 2018

Table 11: Comparison of total fatty acid analysis, EPA content, and DHA content of *P. tricornutum* cultures between the present study and other relevant literature. Fatty acid content for all studies determined using GC with FAME standards.

TFAC (% dry weight)	EPA (% of TFAC)	DHA (% of TFAC)	*P. tricornutum* strain	Study
19-26	6-7	5-6	CCMP 2561	Present study
7-11	10-21	0.3-0.9	Unidentified	Siron et al. 1989
18-31	9-10		UTEX 640	Yongmanitchai et al. 1991
	30-32.5	4-9	UTEX 640	Yongmanitchai et al. 1992
7.6-14.5	23-31	0.45-0.8	Unidentified	Qiao et al. 2016
32	15	2.6	Unidentified	Yang et al. 2017

Table 12: Comparison of maximum growth rate (μ_{max}) of *P. tricornutum* cultures between the present study and other relevant literature. Growth rates were calculated using cell counts or cell count equivalents.

μ_{max} (days^{-1})	*P. tricornutum* strain	μ_{max} (days^{-1})
0.61-0.77	CCMP 2561	Present study
0.4-0.6	UTEX 640	Yongmanitchai et al. 1992
0.97	CCMP 2561	De Martino et al. 2007
0.8-1.2	CCMA 106	Li et al. 2012
1.25-1.5	CCMA 106	Li et al. 2014
0.96-1.61	Unidentified	Qiao et al. 2016

VII. LIST OF APPENDICIES

Appendix 1: Cell counts, optical density, and chlorophyll *a* content for *P. tricornutum* cultures co-cultured with individual bacterial strains and an axenic *P. tricornutum* culture on day 6. Cultures where grown in f/2 media at 25°C on a 12:12 hour light:dark cycle at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Cell counts made using a hemocytometer. OD was measured using a Hach DR 6000 system. Chl-*a* content determined using DAD detection on a HP 1100 HPLC system. Means and standard deviations given for replicates (n=3). Cultures with the same letters are not significantly different from other cultures in the same column.

Culture	Cell Counts (cells/ml)	OD (655 nm)	OD (750 nm)	Chl *a* (ng)
Axenic	1,395,508 ± 69,478[a]	0.027 ± 0.002[a]	0.03 ± 0.003[a]	36.89 ± 15.93[a]
Oceanicaulis 4D	795,534 ± 87,024[b]	0.014 ± 0.003[b]	0.019 ± 0.005[b]	20.00 ± 2.47[a]
Oceanicaulis 6D	741,536 ± 94,597[b]	0.011 ± 0.002[b]	0.017 ± 0.001[b]	19.55 ± 3.99[a]
Oceanicaulis 13A	822,666 ± 24,484[b]	0.017 ± 0.003[b]	0.020 ± 0.002[b]	25.08 ± 4.64[a]

Appendix 2: Cell counts, optical density, and chlorophyll *a* content for *P. tricornutum* cultures co-cultured with individual bacterial strains and an axenic *P. tricornutum* culture on day 20. Cultures where grown in f/2 media at 25°C on a 12:12 hour light:dark cycle at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Cell counts made using a hemocytometer. OD was measured using a Hach DR 6000 system. Chl-*a* content determined using DAD detection on a HP 1100 HPLC system. Means and standard deviations given for replicates (n=3). Cultures with the same letters are not significantly different from other cultures in the same column.

Culture	Cell Counts (cells/ml)	OD (655 nm)	OD (750 nm)	Chl *a* (ng)
Axenic	4,286,666 ± 375,554[b]	0.153 ± 0.026[a]	0.152 ± 0.026[a]	60.14 ± 33.70[a]
Oceanicaulis 4D	4,493,333 ± 212,665[b]	0.159 ± 0.011[a]	0.153 ± 0.014[a]	31.62 ± 11.99[a]
Oceanicaulis 6D	4,128,000 ± 325,617[b]	0.116 ± 0.021[b]	0.157 ± 0.022[a]	27.60 ± 10.34[a]
Oceanicaulis 13A	4,980,000 ± 197,180[a]	0.121 ± 0.021[a]	0.118 ± 0.025[b]	23.07 ± 12.56[a]

Appendix 3: Optical density (OD) and growth rates for *P. tricornutum* co-cultures and axenic culture in growth experiment 1. Cultures where grown in f/2 media at 25°C on a 12:12 hour light:dark cycle at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. OD was measured at 655 nm using a Hach DR 6000 system. Means and standard deviations given for replicates. Cultures with the same letters are not significantly different from other cultures in the same column.

Culture	Maximum OD	OD Day 6	Maximum growth rate (μ_{max})	Exponential growth rate (μ)
Axenic	0.24 ± 0.01^{a}	0.14 ± 0.01^{a}	0.47 ± 0.02^{a}	0.33 ± 0.01^{b}
Loktanella 4BL	0.21 ± 0.02^{b}	0.13 ± 0.01^{ab}	0.50 ± 0.03^{a}	0.32 ± 0.02^{b}
Oceanicaulis 4D	0.24 ± 0.03^{a}	0.13 ± 0.02^{ab}	0.58 ± 0.02^{a}	0.35 ± 0.04^{b}
Rhodobacteria 6CLA	0.18 ± 0.01^{bc}	0.11 ± 0.01^{b}	0.50 ± 0.05^{a}	0.34 ± 0.02^{b}
Oceanicaulis 13A	0.22 ± 0.02^{ab}	0.13 ± 0.01^{ab}	0.50 ± 0.09^{a}	0.38 ± 0.01^{a}
Labrenzia 13C1	0.19 ± 0.02^{bc}	0.12 ± 0.01^{ab}	0.50 ± 0.03^{a}	0.35 ± 0.03^{b}
Marinobacter 19DW	0.19 ± 0.01^{bc}	0.08 ± 0.02^{c}	0.30 ± 0.14^{b}	0.26 ± 0.05^{c}
Devosia EAB7W2	0.24 ± 0.02^{a}	0.15 ± 0.01^{a}	0.54 ± 0.02^{a}	0.36 ± 0.02^{b}
Stappia ARW1T	0.22 ± 0.10^{a}	0.13 ± 0.01^{ab}	0.53 ± 0.05^{a}	0.35 ± 0.01^{b}
Algoriphagus ARW1R1	0.24 ± 0.01^{a}	0.13 ± 0.02^{ab}	0.50 ± 0.06^{a}	0.37 ± 0.01^{b}
Muricauda ARW1Y1	0.19 ± 0.01^{b}	0.11 ± 0.01^{b}	0.49 ± 0.03^{a}	0.33 ± 0.03^{b}
Arenibacter ARW7G5Y1	0.18 ± 0.01^{bc}	0.11 ± 0.01^{b}	0.45 ± 0.02^{a}	0.31 ± 0.05^{b}
Alcanivorax EA2	0.20 ± 0.02^{b}	0.11 ± 0.01^{b}	0.43 ± 0.07^{a}	0.32 ± 0.04^{b}
Alcaligenaceae EA3	0.20 ± 0.01^{b}	0.12 ± 0.01^{ab}	0.49 ± 0.06^{a}	0.39 ± 0.01^{a}
Alphaproteobacteria EA10	0.22 ± 0.01^{a}	0.13 ± 0.01^{ab}	0.51 ± 0.05^{a}	0.34 ± 0.01^{b}
Marinobacter 3-2	0.21 ± 0.01^{b}	0.13 ± 0.01^{ab}	0.49 ± 0.09^{a}	0.36 ± 0.02^{b}

Appendix 4: Optical density (OD) and growth rates for *P. tricornutum* co-cultures and axenic culture in growth experiment 2. Cultures where grown in f/2 media at 25°C on a 12:12 hour light:dark cycle at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. OD was measured at 655 nm using a Hach DR 6000 system. Means and standard deviations given for replicates. Cultures with the same letters are not significantly different from other cultures in the same column.

Culture	Maximum OD	OD Day 6	Maximum growth rate (μ_{max})	Exponential growth rate (μ)
Axenic	0.19 ± 0.023^{b}	0.12 ± 0.01^{b}	0.50 ± 0.01^{a}	0.34 ± 0.02^{a}
Loktanella 4BL	0.19 ± 0.03^{b}	0.12 ± 0.02^{b}	0.45 ± 0.07^{a}	0.30 ± 0.02^{a}
Oceanicaulis 4D	0.22 ± 0.05^{b}	0.13 ± 0.02^{b}	0.48 ± 0.08^{a}	0.35 ± 0.01^{a}
Rhodobacteria 6CLA	0.20 ± 0.03^{b}	0.12 ± 0.01^{b}	0.44 ± 0.02^{a}	0.34 ± 0.02^{a}
Oceanicaulis 13A	0.20 ± 0.02^{b}	0.12 ± 0.01^{b}	0.50 ± 0.07^{a}	0.32 ± 0.03^{a}
Labrenzia 13C1	0.22 ± 0.02^{b}	0.13 ± 0.01^{b}	0.50 ± 0.03^{a}	0.37 ± 0.03^{a}
Marinobacter 19DW	0.20 ± 0.02^{b}	0.10 ± 0.03^{bc}	0.48 ± 0.05^{a}	0.36 ± 0.09^{a}
Devosia EAB7W2	0.23 ± 0.01^{ab}	0.13 ± 0.01^{b}	0.53 ± 0.01^{a}	0.38 ± 0.01^{a}
Stappia ARW1T	0.19 ± 0.01^{b}	0.12 ± 0.01^{b}	0.47 ± 0.02^{a}	0.35 ± 0.08^{a}
Algoriphagus ARW1R1	0.25 ± 0.02^{a}	0.15 ± 0.01^{a}	0.56 ± 0.01^{a}	0.39 ± 0.02^{a}
Muricauda ARW1Y1	0.20 ± 0.01^{b}	0.12 ± 0.01^{b}	0.56 ± 0.06^{a}	0.36 ± 0.02^{a}
Arenibacter ARW7G5Y1	0.21 ± 0.02^{b}	0.13 ± 0.01^{b}	0.55 ± 0.01^{a}	0.34 ± 0.02^{a}
Alcanivorax EA2	0.20 ± 0.01^{b}	0.12 ± 0.01^{b}	0.52 ± 0.02^{a}	0.35 ± 0.02^{a}
Alcaligenaceae EA3	0.23 ± 0.01^{ab}	0.15 ± 0.01^{ab}	0.58 ± 0.02^{a}	0.39 ± 0.02^{a}
Alphaproteobacteria EA10	0.21 ± 0.01^{b}	0.13 ± 0.01^{b}	0.54 ± 0.04^{a}	0.36 ± 0.01^{a}
Marinobacter 3-2	0.23 ± 0.01^{ab}	0.14 ± 0.01^{b}	0.56 ± 0.04^{a}	0.39 ± 0.01^{a}

Appendix 5: Optical density (OD) and growth rates for *P. tricornutum* co-cultures and axenic culture in growth experiment 3. Cultures where grown in f/2 media at 25°C on a 12:12 hour light:dark cycle at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. OD was measured at 655 nm using a Hach DR 6000 system. Means and standard deviations given for replicates. Cultures with the same letters are not significantly different from other cultures in the same column.

Culture	Maximum OD	OD Day 6	Maximum growth rate (μ_{max})	Exponential growth rate (μ)
Axenic	0.20 ± 0.02^{b}	0.11 ± 0.02^{ab}	0.39 ± 0.10^{b}	0.31 ± 0.02^{ab}
Loktanella 4BL	0.22 ± 0.01^{ab}	0.14 ± 0.01^{a}	0.47 ± 0.08^{ab}	0.33 ± 0.01^{a}
Oceanicaulis 4D	0.23 ± 0.02^{ab}	0.11 ± 0.04^{ab}	0.44 ± 0.08^{b}	0.34 ± 0.04^{a}
Rhodobacteria 6CLA	0.21 ± 0.01^{b}	0.13 ± 0.01^{a}	0.47 ± 0.02^{ab}	0.34 ± 0.01^{ab}
Oceanicaulis 13A	0.22 ± 0.02^{ab}	0.12 ± 0.03^{ab}	0.42 ± 0.05^{b}	0.32 ± 0.03
Labrenzia 13C1	0.22 ± 0.01^{ab}	0.13 ± 0.01^{a}	0.42 ± 0.02^{b}	0.33 ± 0.01^{a}
Marinobacter 19DW	0.22 ± 0.01^{ab}	0.11 ± 0.02^{ab}	0.38 ± 0.05^{b}	0.34 ± 0.03^{a}
Devosia EAB7W2	0.24 ± 0.01^{a}	0.15 ± 0.02^{a}	0.46 ± 0.04^{b}	0.33 ± 0.05^{a}
Stappia ARW1T	0.20 ± 0.01^{b}	0.13 ± 0.01^{a}	0.47 ± 0.01^{ab}	0.33 ± 0.02^{a}
Algoriphagus ARW1R1	0.21 ± 0.01^{b}	0.13 ± 0.01^{a}	0.49 ± 0.03^{a}	0.35 ± 0.01^{a}
Muricauda ARW1Y1	0.18 ± 0.01^{bc}	0.10 ± 0.02^{b}	0.46 ± 0.06^{b}	0.29 ± 0.01^{b}
Arenibacter ARW7G5Y1	0.21 ± 0.02^{b}	0.13 ± 0.02^{a}	0.47 ± 0.01^{ab}	0.31 ± 0.05^{ab}
Alcanivorax EA2	0.20 ± 0.01^{b}	0.13 ± 0.01^{a}	0.41 ± 0.05^{b}	0.31 ± 0.01^{ab}
Alcaligenaceae EA3	0.22 ± 0.01^{ab}	0.12 ± 0.01^{ab}	0.48 ± 0.07^{ab}	0.33 ± 0.02^{a}
Alphaproteobacteria EA10	0.21 ± 0.01^{b}	0.11 ± 0.01^{ab}	0.42 ± 0.08^{b}	0.28 ± 0.06^{b}
Marinobacter 3-2	0.23 ± 0.01^{a}	0.14 ± 0.01^{a}	0.51 ± 0.01^{a}	0.35 ± 0.02^{a}