jmb

Assessment of the Dynamics of Microbial Community Associated with *Tetraselmis suecica* Culture under Different LED Lights Using Next-Generation Sequencing

Su-Jeong Yang[1†], Hyun-Woo Kim[1†], Seok-Gwan Choi[2], Sangdeok Chung[2], Seok Jin Oh[3], Shweta Borkar[4], and Hak Jun Kim[4*]

[1]*Interdisciplinary Program of Biomedical, Mechanical and Electrical Engineering, Pukyong National University, Busan 48513, Republic of Korea*

[2]*National Institute of Fisheries Science (NIFS), Busan 46083, Republic of Korea*

[3]*Department of Oceanography, Pukyong National University, Busan 48513, Republic of Korea*

[4]*Department of Chemistry, Pukyong National University, Busan 48513, Republic of Korea*

Tetraselmis is a green algal genus, some of whose species are important in aquaculture as well as biotechnology. In algal culture, fluorescent lamps, traditional light source for culturing algae, are now being replaced by a cost-effective light-emitting diodes (LEDs). In this study, we investigated the effect of LED light of different wavelengths (white, red, yellow, and blue) on the growth of *Tetraselmis suecica* and its associated microbial community structures using the next-generation sequencing (NGS). The fastest growth rate of *T. suecica* was shown in the red light, whereas the slowest was in yellow. The highest OTUs (3426) were identified on day 0, whereas the lowest ones (308) were found on day 15 under red light. The top 100 OTUs associated with day 0 and day 5 cultures of *T. suecica* under the red and yellow LED were compared. Only 26 OTUs were commonly identified among four samples. The highest numbers of unique OTUs were identified at day 0, indicating the high degree of initial microbial diversity of the *T. suecica* inoculum. The red light-unique OTUs occupied 34.98%, whereas the yellow-specific OTUs accounted for only 2.2%. This result suggested a higher degree of interaction in *T. suecica* culture under the red light, where stronger photosynthesis occurs. Apparently, the microbial community associated with *T. suecica* related to the oxygen produced by algal photosynthesis. This result may expand our knowledge about the algae-bacteria consortia, which would be useful for various biotechnological applications including wastewater treatment, bioremediation, and sustainable aquaculture.

Keywords: Next-generation sequencing, metagenomics, LED, microalgae, microbial community

Introduction

Tetraselmis is a green algal genus, some of whose species are widely aquacultured as good food sources for bivalves, penaeid shrimp larvae, and rotifers [1]. These marine microalgae are known to have a large spectrum of antimicrobial activity [2, 3] along with high potential as a probiotic for fish [4]. *Tetraselmis* has also been proposed as a source of vitamins or fatty acids for humans and animals [5–7]. In addition, mass culturing of *Tetraselmis* is essential for biofuel production and has been carried out in various studies [8, 9]. As one of the cost-effective algal culture system, the traditional fluorescent lamps are now being replaced by a light-emitting diodes (LEDs) for its longer lifespan and higher electrical efficiency

Since microalgae are photosynthetic organisms, light is the most important factor in culturing them. Many studies on algae were performed to understand the wavelength

dependence of the photosynthesis process, while the effect of the exposure to different light spectra has been much less investigated [10]. Microalgae require optimal light conditions to achieve maximum photosynthetic rate economically [11]. Located at the bottom of the aquatic food chain, microalgae are autotrophic organisms that produce organic matter through photosynthesis. They form colonies or are surrounded by a microbiome of mucous substances called the 'phycosphere,' where many bacteria are attached and symbiotic. Strong interactions between microalgae and bacteria occur in the phycosphere and can have either a positive or negative effect on each other. Bacteria provide microalgae with CO_2, inorganic nutrients, and vitamin sources, but obtain the oxygen and extracellular substances generated by microalgae. Microalgae and bacteria exist together in almost all aquatic environments and play key roles in nutrient cycling and energy flow [12, 13].

Microbial communities are usually examined by morphological identification. However, since the bacterial communities are difficult to observe directly, they are first separated by isolation and then analyzed using methods such as clone libraries or RFLP [14, 15], which requires a lot of time and effort. Next-generation sequencing (NGS) is a technique used to quickly analyze microbiota in detail without isolating the microorganism separately [16, 17]. There are two ways; the first is a nucleotide sequence analysis targeting 16S rRNA and the second is a shotgun method. We chose the former, using DNA barcodes. The 16S rRNA has a conserved region common to all species and a hypervariable region which is used as a DNA barcode that can classify specific species. With NGS, metagenomic analysis makes it possible to identify all species in a sample, even those that are present in low abundance. The Illumina Miseq platform in particular generates a large number of high-quality sequence reads that enable detection of the microbial taxa across a high number of taxonomic profiles from samples [18].

It was confirmed that the growth of microalgae varied depending on different wavelengths [11, 19]. As a result, interacting microorganisms are affected by wavelength or the rate of photosynthesis of algae. However, little research has been done on the effect of light wavelengths on the algae-microbiome community structure. In this study, we investigated the effect of LED light of different wavelengths (white, red, yellow, blue) on the growth rate of *T. suecica* and its associated microbial communities using next-generation sequencing (NGS) technology.

Materials and Methods

Culture

The experimental species *T. suecica* was received from the Library of Marine Samples, KIOST, Korea. For the culture, f/2 medium was used, which was filtered (0.22 µm pore size, Millipore GSWP) with selenium to a final concentration of 0.001 µM. The medium was maintained at 20°C, 30 psu, with light intensity of about 100 µmol/m^2/s (12L: 12D; cool-white fluorescent lamp). The experimental instruments were UV-treated for 20 min or more, and all experiments were performed on a clean bench.

As a light source, a fluorescent lamp with multiple wavelengths, a red light (650 nm), a yellow light (590 nm), and a blue light (450 nm) were used. The microalgae were grown to a late stage of the logarithmic growth phase under a cool-white fluorescent lamp (12L: 12D) at 20°C, 30 psu and then inoculated to a final density of cells with about 1.0×10^2 in f/2 medium 3L [20]. For each condition, two bottles were used.

Genomic DNA Extraction

From the mass cultured samples, 15 ml was filtered through a 0.45 µM GN-6 membrane filter (Pall Corporation, USA) every 5 days until the 25th day. Next, the filters were homogenized with a TissueLyser II (QIAGEN, Germany) and incubated in a heat block at 60°C for 2 h. Then, genomic DNA was extracted using a DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's instructions and stored at −70°C until used as a template for qPCR and library preparation.

Quantitative PCR Analysis of *T. suecica*

Genomic DNA was quantified by quantitative real-time PCR (qPCR) with phytoplankton primers P23MISQF1 and P23MISQR1 targeting plastid 23S ribosomal DNA and 341F and 785R targeting 16S ribosomal DNA to measure the relative number of *T. suecica* and microbial community, respectively (Table 1). The PCR mixture (20 µl) contained 4 µl ultrapure water, 1 µl forward and reverse primer (10 µM)), 10 µl 2X SYBR Green Premix Ex Taq II (Takara Bio Inc., Japan) and 4 µl genomic DNA as a template. Real-time PCR was carried out under the following conditions: initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec. The process was completed with a final extension at 72°C for 5 min. A standard curve was constructed using plasmid containing each primer set with a range of 0.01 pg to 0.01 ng [21, 22]. The reaction was performed using a DNA Engine Chromo 4 Real-Time PCR Detection System (Bio-Rad, USA).

NGS Library Preparation and Sequencing

In order to analyze the microbial community structures associated with the growth of *T. suecica*, cultures at day 5 and day 15 under the red and yellow LED lights were harvested and

Table 1. Primers used in this study.

Name	Sequence (5′ to 3′)	Target region
341F	CCTACGGGNGGCWGCAG	16S
785R	GACTACHVGGGTATCTAATCC	16S
P23MISQF1	GGACARWAAGACCCTATGMAG	23S
P23MISQR1	AGATYAGCCTGTTATCCCT	23S
Forward adaptor	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	
Reverse adaptor	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	

samples at day 0 were used as a control. The first PCR was conducted using 341F and 785R with overhanging adaptors for MiSeq Sequencing (Table 1). The PCR mixture (20 μl) contained 1 μl primer (10 μM, forward and reverse), 0.1 μl Ex Taq DNA polymerase (TaKaRa), 2 μl Ex 10X Buffer, 2 μl dNTPs (0.5 μM, TaKaRa), ultrapure water and a template. The amounts of ultrapure water and template were adjusted to have the same DNA concentrations for the library construction as well as the samples. The PCR was performed under the following conditions: initial denaturation step at 94°C for 3 min, followed by 10 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec. The process was completed with a final extension at 72°C for 3 min. The first PCR products were separated by 1.5% agarose gel electrophoresis and stained with loading star dye (Dynebio, Korea). Amplicons with the expected size (511 bp) were cut from the gel and purified using an AccuPrep Gel Purification Kit (Bioneer, Korea) for the second PCR, which was performed using Illumina Nextera XT indexing primers (Table 1). The PCR mixture (20 μl) contained 9.3 μl of ultrapure water, 1 μl primer (10 μM, forward and reverse), 0.5 μl Phusion High Fidelity DNA polymerase (NEB, UK), 4 μl 5X Phusion Buffer, 0.5 μl dNTPs (10 μM, TaKaRa) and 4 μl purified first PCR products as a template and the PCR was carried out under the following conditions: initial denaturation step at 94°C for 3 min, followed by 15 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec. The process was completed with a final extension at 72°C for 3 min. Then, gel purification was performed as described above. The quality and quantity of the libraries were measured using a 2100 Bioanalyzer (Agilent Technologies, USA) and sequencing was performed at 300 bp paired-end reads with a MiSeq sequencer (Illumina, USA).

Bioinformatics of NGS Data

Among the raw data, sequences with under QV 20 and read length less than 100 nucleotides were trimmed using the CLC Genomic Workbench v.8.0 (CLC Bio, USA). End-paired amplicons were constructed with over 6 bp overlapping sequences and omitting any mismatches option, size selection (400~500 bp), and primer trimming with the pdiffs=1 option were performed using Mothur software v.1.35.0 [23]. Operational taxonomic units with 99.6% similarity cutoff were clustered and chimeras were

removed using UCHIME software v.8.1 (http://drive5.com/uchime). OTUs with less than 0.001% of total reads were removed. The sequences of OTUs were compared against known species from the NCBI-NT database using BLAST (BLASTN, version 2.2.30+). OTU sequences with more than 97% identity to the database were assigned to the top-hit species names while genus names were assigned for OTUs with 90~96% identity. OTUs with less than 90% identity to the database were described as "unclassified." The Venn diagram was generated with Draw Venn Diagram (http://bioinformatics.psb.ugent.be /webtools/Venn/).

Results

Quantitative Analysis of Microbial Community

According to qPCR result with P23MISQF1 and P23MISQR1 primer set, the fastest growth rate of *T. suecica* was identified under the red LED light followed by white, blue and yellow (Fig. 1A). The rate was steadily increased in all wavelengths during the study period, but not in the red wavelength on day 25. The 341F and 785R primer is a universal primer set that amplifies the 16S rRNA region of microorganisms. The number of microorganisms increased steeply after one day of cultivation, reaching a peak and showing fast growth rate compared with *T. suecica* (Fig. 1B). Thereafter, microbial copy numbers did not show any significant changes, indicating the limit of nutrition in the culture. It was noteworthy that the lowest copy numbers of total microorganism were also shown in the culture under the yellow light throughout the culture time. This may have come from the low amount of organic carbon source by *T. suecica* under the light. As shown in Fig. 1C, the ratio of *T. suecica* to total bacteria was low until day 10 of inoculation, but this ratio increased significantly thereafter. (Fig. 1C). This result indicated the exponential growth of *T. suecica* approximately 10 days after inoculation.

Community Structures Generated by Next-Generation Sequencing

Microbial community structures associated with the growth rates of *T. suecica* were analyzed by a next-generation
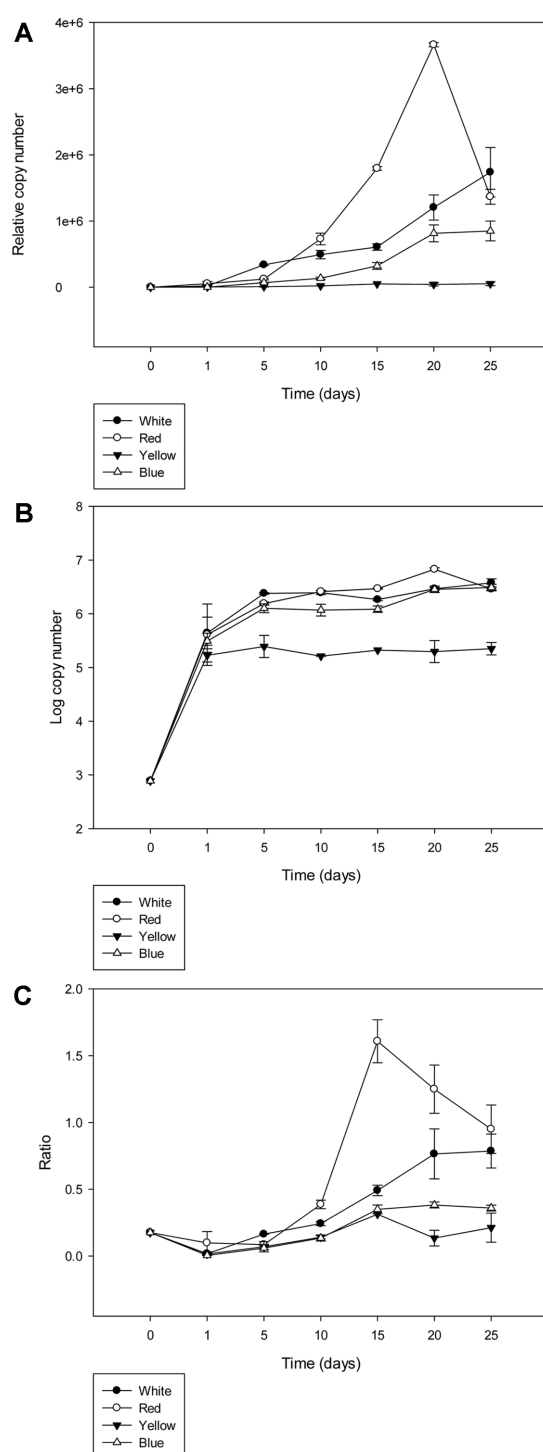
Fig. 1. Quantitative PCR result of *Tetraselmis suecica* and its associated microorganisms grown under the four different wavelength LED lights.

(**A**) The relative copy numbers of *T. suecica* measured by the plastid 23S rDNA. (**B**) The relative copy numbers of microorganisms by the numbers of 16S rRNA. (**C**) The ratio of *T. suecica* to total bacteria in different cultivation times.

sequencing (NGS) technique using 341F and 785R primer. The cultures on days 5 and 15 under the red light were chosen as 'fast-growing' microbial communities, whereas those under the yellow light were named 'slow-growing' communities. The day 0 community was analyzed as the control. All OTUs were assigned to species if the similarity was 97% or more, to genus if similarity was 90−96%, and to unclassified if less than 90%. Most OTUs showed more than 97% sequence identity to the database. The obtained OTUs numbers ranged from 308 to 3426. The highest OTU numbers were identified on day 0 (3426), whereas the lowest ones were found on day 15 under the red light (15R). For our convenience, OTUs with lower than 0.001% of total reads were eliminated from further analysis, which occupied approximately 2–4% of total read numbers. Obtained OTUs were classified into 1 phytoplankton phylum including Chlorophyceae and 8 bacterial classes including Actinobacteridae, Alphaproteobacteria, Cytophagia, Flavobacteria, Gammaproteobacteria, Phycisphaerae, Planctomycetacia, and Sphingobacteria (Table 2). Smaller class numbers were identified in the red lights (17 on day 15 and 21 on day 5), whereas its higher numbers were found in the yellow light (29 on both day 15 and day 5). Day 0 showed the highest microbial class numbers. Collectively, lower microbial taxa were identified in the 'fast-growing' *T. suecica* cultures, suggesting strong photosynthesis has an adverse effect in several microbial classes such as Planctomycetia, which was identified only in the yellow light (Table 2).

Microbial community structures with different culture times and lights were compared (Table 3 and Fig. 2). *T. suecica* dominated in all the examined cultures except for day 5 under yellow light (5Y). On day 0, *T. suecica* occupied about 34% followed by *M. roseacus* (30%), *Rhabdobacter* sp (8%), and *Halomonas* sp. (7%). In 5R, *T. suecica* accounted for about half of the community, 21% *Halomonas* sp., 17% *Alteromonas australica*, 6% *M. roseacus*. In day 5 under the yellow light (day 5Y), *M. roseacus* occupied 38% followed by 11% of *Pseudomonas* sp., and 6% of *Rhabdobacter* sp. On day 15, *M. roseacus* accounted for 37% and 33% in the red and yellow lights, respectively. *M. roseacus* was dominant compared to other bacteria (Fig. 2A). Except for *T. suecica*, the community structure of 5R appeared different than 5Y compared to the day of inoculation. On day 15, there were only a small number of bacteria that were present on day 5, and *M. roseacus* was most dominant. (Fig. 2B).

Microbial community structures associated with *T. seucica* cultures under the LED lights with two different wavelengths were compared (Fig. 3). First, we compared the top

Table 2. Summary of OTUs generated by 16S universal primers at day 0, 5, and 15 samples under the red and yellow lights.

Class	Day 0		Day 5 - Red		Day 5 - Yellow		Day 15 - Red		Day 15 - Yellow	
	OTUs	Proportion (%)	OTUs	Proportion (%)	OTUs	Proportion (%)	OTUs	Proportion (%)	OTUs	Proportion (%)
Actinobacteridae	1	1.72								
Alphaproteobacteria	13	36.32	7	7.15	12	44.21	7	38.78	14	38.20
Chlorophyceae	1	34.23	1	50.08	1	23.21	1	55.38	1	54.33
Cytophagia	2	8.15			1	6.21	1	0.89	1	1.58
Flavobacteriia	1	0.12	1	0.26			1	0.22		
Gammaproteobacteria	18	18.09	10	41.05	11	22.35	5	2.77	7	3.05
Phycisphaerae	1	1.14	1	1.25	1	2.57	1	1.57	1	1.46
Planctomycetia					1	0.99			2	0.63
Sphinogobacteriia	1	0.23	1	0.22	1	0.33	1	0.39	2	0.50
Unclassified					1	0.14			1	0.25
Total	38	100.00	21	100.00	29	100.00	17	100.00	29	100.00

100 OTUs among day 0, day 5 under the yellow (5Y) and red (5R) using the Venn-diagram (Fig. 3A). Only 26 OTUs were commonly identified among day 0, 5R, and 5Y. The highest numbers of unique OTUs were identified at day 0 indicating the high degree of initial microbial diversity of the *T. suecica* inoculum. The highest proportion of unique OTUs were identified in the red light (34.98%), whereas the yellow-specific OTUs occupied only 2.2% (Table 4-1) This result suggested that a higher degree of interaction in *T. suecica* culture under the red light, in which its strong photosynthesis occurs. By contrast, the highest proportion of shared OTUs with those in day 0 were identified in 5Y (19%) compared with those in 5R (Table 4-1 and Fig. 3A).

On day 15, compared with the remaining OTUs in the red and yellow wavelengths, the numbers of shared OTU were 37, but the shared proportions were 97.31% and 91.2%, respectively, in which *M. roseacus* was the most abundant bacteria (Table 4-2). Duplicated experiments were conducted to determine the microbiota associated with the growth of photosynthetic *T. seucica* (Figs. 3C and 3D). On day 15, the microbial communities under the red light exhibited a high degree of similarity sharing 53 OTUs with 98.56% and 97.93% identity in their proportions. By contrast, microbial communities in duplicated experiments under the yellow light showed higher degree of dissimilarity, sharing only 13 OTUs with 62.73% and 3.89% identity (Fig. 3D). This result suggested that the microbial community associated with *T. suecica* is strongly related to photosynthesis.

The reproducibility of the microbial community associated with *T. suecica* was tested by the replication of the experiment (Table 4-3,4). We found that 53 OTUs shared between two independent cultures under the red light on

day 5 occupied 99% and 98%, respectively (Fig. 3C). Although the numbers of unique OTUs in each culture under the red light were 43 (5R-1) and 41 (5R-2), their proportions were negligible, indicating that the microbial community associated with the growth of *T. suecica* was not a result of random combination of bacterial components. For the slow growth *T. suecica* under the yellow light, which exhibited the low photosynthetic efficiency, only 13 OTUs were shared and their proportions were 63% and 96%, respectively, depending on the growth of *T. suecica* (Table 4-3 and Fig. 3C). Collectively, on day 5, the efficiency of photosynthesis of *T. suecica* may have been the critical factor in determining microbial community.

Discussion

In this study, we investigated the effect of the LED light with different wavelengths (white, red, yellow, blue) on the growth rate of *T. suecica* and its associated microbial communities using next-generation sequencing (NGS) technology. Analysis of microbial community structure using NGS techniques revealed significantly higher diversity than the traditionall methods including the clone libraries or DGGE community profiling [24]. The primer set used in the present study, 341F and 785R, has been proven to show the wide range of microbial taxa coverage with little non-specific amplification [25, 26]. The results of artificial conditions in the laboratory are essential to understanding the growth characteristics in outdoor cultures [27]. The culture was non-axenic, which is associated with bacteria present in the natural environment and cultured with algae [16]. Besides, the non-axenic microalgae cultures

A

B
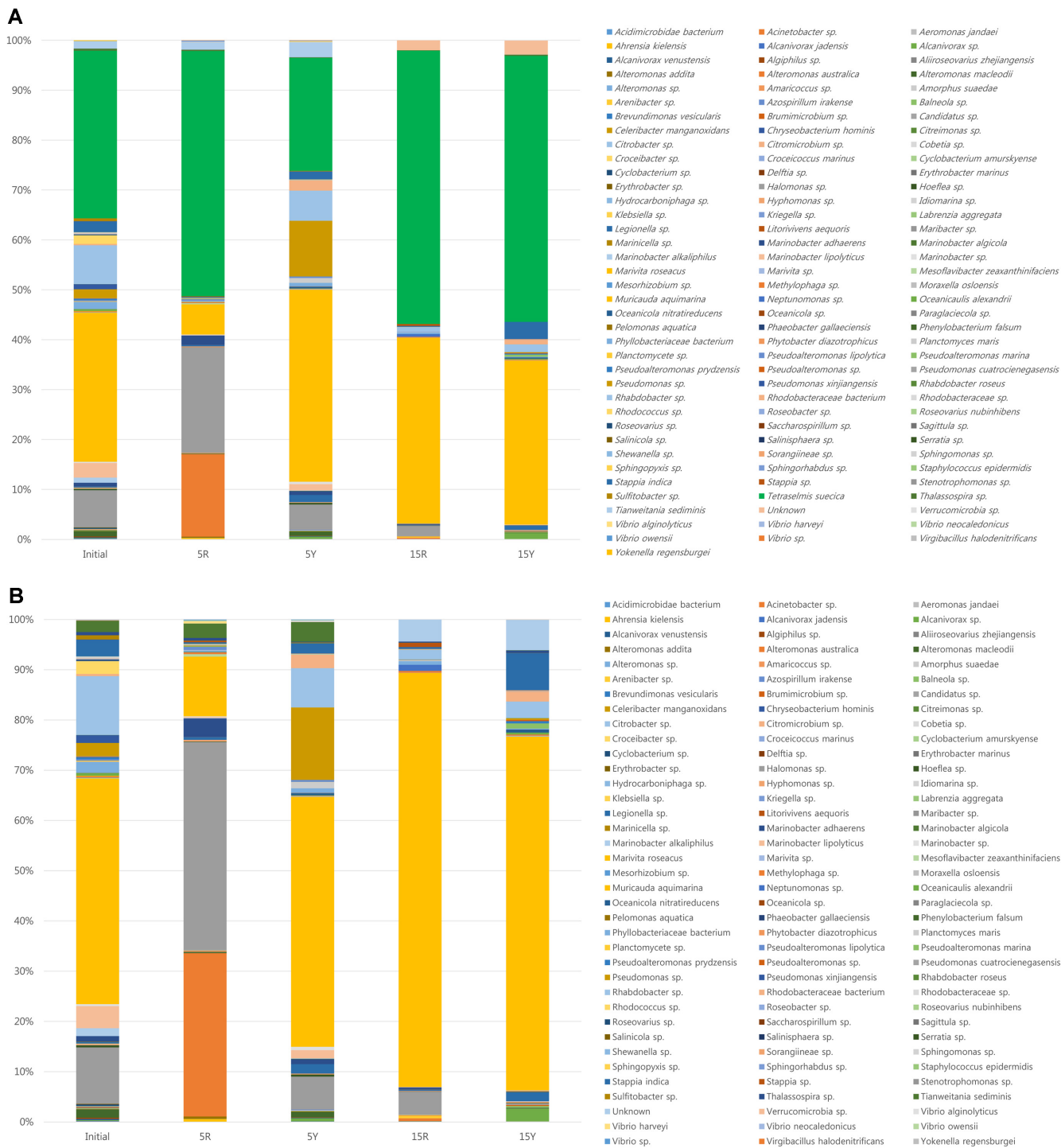
Fig. 2. Microbial community structure at species level on day 0, day 5 of the red and yellow wavelengths with *T. suecica* included (**A**) and excluded (**B**).

Each bar shows the proportion of microbial species according to 97 % sequence similarity.

are applied in the green-water technique, and it is important to recognize the microbiota associated with the microalgae cultures [28].

A light regime generally produces the most intense photosynthesis at wavelength from 575 to 720 nm, and is most effective in the red (650~680 nm) and blue (400~500 nm).

Fig. 3. Three-way Venn diagram illustrating the number of shared and specific OTUs among the day of inoculation and day 5 (**A**), between the red and yellow wavelengths on day 15 (**B**), between same wavelengths of red light (**C**), and yellow light (**D**).

According to the previous study, the growth rate of *T. suecica* under different types of wavelengths was reported to be fast in order of white, red and blue [29], but in this study, red had the fastest growth rate followed by white, blue, and yellow. 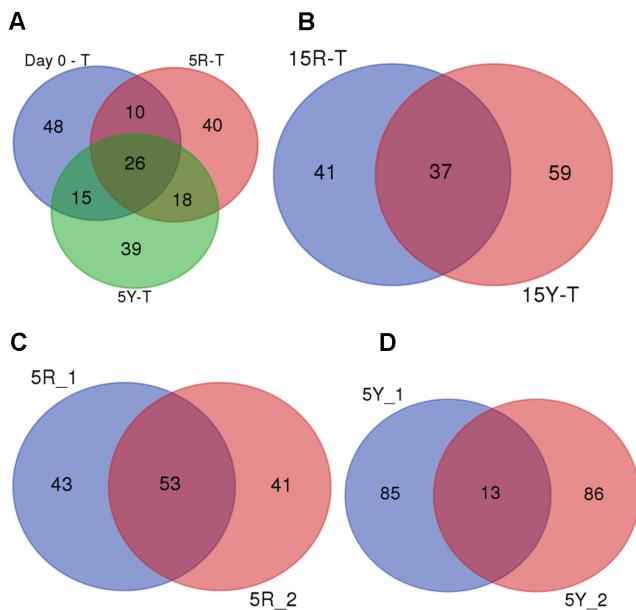This result may be due to the difference in the white light source. The spectrums of commercially available white light lamps are different and the white fluorescent lamp in this study may not be effective as one in another study. Further study should be conducted to compare white lamps with different spectrums. The samples cultured until the 25th day seemed to reach the stationary phase and the red wavelength with the fastest growth seemed to be the death phase from the 15th day presumably due to the nutritional limit in the culture (Fig. 1).

After a day of inoculation, the ratio of *T. suecica* to bacteria is low because bacterial growth is much faster than eukaryotic microalgae consuming the organic nutrients in the subculture, but soon its growth rate is decreased by the limited amount of the organic nutrient before the growth of *T. suecica*, which further provides it by photosynthesis (Fig. 3). In fact, we have found that *T. suecica* was the least abundant among the samples at the slowest growing yellow wavelength (Fig. 4). This appears to be due to the relatively low photosynthesis of algae at the yellow

wavelength providing the low amounts of organic matter for bacterial use.

While the proportion of other bacteria decreased over time, only *M. roseacus* increased in the culture of *T. suecica* (Fig. 4). *Marivita* is a heterotrophic and strictly aerobic bacterium, which is closely associated with the algae [30]. Therefore, given the high number of *T suecica*, the higher amount of the produced oxygen may have facilitated the growth of this bacterium. *M. roseacus* is a novel species that was first reported to be isolated from the surface of a coccolithophore. It appears to be beneficial to the growth of algae, which may exhibit specific dependence between bacteria and algae even though the genus *Marivita* may be a product induced by artificial laboratory culture experiments [30]. Probably because of its special metabolic pathway to algae, it may occupy half of the community composition on day 15 and have growth priority compared to other bacteria. So, *M. roseacus* seemed to be predominant despite the different wavelengths (Fig. 2). Alternatively, *M. roseacus* is known to produce bacteriochlorophyll *a*, which can survive by photosynthesis in conditions with limited organic carbon sources [31]. Certain bacteria have been reported to exert an effect on microalgae, indicating that the combination of microalgae and bacteria may be decisive for co-culture [12].

It is conceivable that bacteria will interact with microalgae with nutrients during the culture. Here, we demonstrated that fast-growing bacteria consumed organic nutrients quickly in the subculture, and metabolites generated by bacterial communities affected the growth of the microalgae as well. However, it is surprising to find out that the remaining bacterial community on day 15 shared about half of its OTUs and its proportion of contigs was more than 90% (Table 4-2 and Fig. 3B). The community structures between the two samples were also highly similar to each other. A similar result was also shown in the study by Han, in which bacterial density remained at a similar level by consuming the original organic substance and resulted in a similar microalgal density [12]. Therefore, the fast-growing bacteria determined the initial microbial community with little effect by the low-numbered microalgae. Although the growth of both microalgae and bacteria is affected by their complex interactions, the initial community composition appears to be determined by the random composition of the inoculated bacteria in the subcultured samples. Thereafter, the determined bacterial community can be harmful or beneficial to the growth of a microalgae affecting its growth. The experiment was duplicated per each condition, and the community structures of two

Table 3. Top 20 OTUs associated with day 0, day 5, and day 15 samples of *T. suecica* under the red and yellow LED lights.

No	Day 0			Day 5 - Red			Day 5 - Yellow			Day 15 - Red			Day 15 - Yellow		
	Species	GenBank number	Proportion (%)	Species	GenBank number	Proportion (%)	Species	GenBank number	Proportion (%)	Species	GenBank number	Proportion (%)	Species	GenBank number	Proportion (%)
1	*Tetraselmis suecica*	KU167097	34.00	*Tetraselmis suecica*	KU167097	50.08	*Marivita roseacus*	NR_132662	38.34	*Tetraselmis suecica*	KU167097	55.38	*Tetraselmis suecica*	KU167097	54.33
2	*Marivita roseacus*	NR_132662	30.15	*Halomonas* sp.	KY474018	20.78	*Tetraselmis suecica*	KU167097	23.21	*Marivita roseacus*	NR_132662	37.26	*Marivita roseacus*	NR_132662	31.99
3	*Rhabdobacter* sp.	NR_147750	7.88	*Alteromonas* sp.	KP099958	16.72	*Pseudomonas* sp.	KY020318	11.38	*Halomonas* sp.	KY474018	2.09	*Stappia indica*	KY616246	2.84
4	*Halomonas* sp.	LT934170	7.55	*Marivita roseacus*	NR_132662	6.01	*Rhabdobacter* sp.	NR_147750	6.21	Unclassified	NR_147750	1.57	*Rhabdobacter* sp.	NR_147750	1.58
5	*Marinobacter lipolyticus*	KX818042	2.96	*Marinobacter adhaerens*	KY601871	1.88	*Halomonas* sp.	KY474018	5.10	*Rhabdobacter* sp.	NR_147750	0.89	Unclassified		1.46
6	*Stappia indica*	MF928372	2.25	Unclassified		1.25	Unclassified		2.57	*Oceanicaulis alexandrii*	FN562402	0.55	*Alcanivorax* sp.	KU681506	1.19
7	*Rhodococcus fascians*	MG205633	1.71	*Halomonas* sp.	KY474018	0.33	*Rhodobacteraceae bacterium*	KM279014	1.79	Unclassified		0.39	*Rhodobacteraceae bacterium*	KM279014	0.83
8	*Phyllobacteriaceae bacterium*	KM279012	1.47	*Ahrensia kielensis*	KM613150	0.32	*Legionella* sp.	KU508793	1.42	*Phyllobacteriaceae bacterium*	KM279012	0.33	*Legionella* sp.	KU508793	0.81
9	*Pseudomonas stutzeri*	MF383463	1.29	*Pseudoalteromonas lipolytica*	LC221845	0.32	*Marinobacter lipolyticus*	KX818042	1.30	*Stappia* sp.	LT629784	0.24	*Marivita roseacus*	NR_132662	0.65
10	*Alteromonas* sp.	MG010445	1.14	*Thalassospira* sp.	LN868390	0.28	*Stappia indica*	LN868390	1.20	*Alteromonas australica*	KP099958	0.23	Unclassified		0.54
11	Unclassified		1.13	*Mesoflavibacter zeaxanthinifaciens*	NR_114033	0.26	*Planctomyces maris*	NR_025327	0.99	*Arenibacter* sp.	KU948154	0.22	*Planctomyces maris*	NR_025327	0.49
12	*Marinobacter alkaliphilus*	MG029454	1.06	*Legionella* sp.	KU508793	0.25	*Alteromonas macleodii*	KY436447	0.90	*Marivita roseacus*	NR_132662	0.18	*Stappia indica*	KY616246	0.40
13	*Pseudomonas xinjiangensis*	KU601273	1.04	Unclassified		0.22	*Marinobacter adhaerens*	KY604871	0.78	*Methylophaga* sp.	KC295343	0.15	Unclassified		0.36
14	*Marinobacter adhaerens*	KY604871	0.76	*Vibrio harveyi*	KY697683	0.22	*Phyllobacteriaceae bacterium*	KM279012	0.70	*Legionella* sp.	KU508793	0.15	Unclassified		0.25
15	*Pseudomonas* sp.	KX000012	0.48	*Alteromonas addita*	KY787160	0.22	*Alcanivorax* sp.	KU681506	0.47	*Marinobacter adhaerens*	KY604871	0.15	*Thalassospira* sp.	LN868390	0.24
16	*Oceanicaulis alexandrii*	FN562403	0.38	*Methylophaga* sp.	KC295343	0.19	*Marinobacter* sp.	LT221258	0.40	*Stappia indica*	KY616246	0.12	*Oceanicola nitratireducens*	NR_116369	0.22
17	*Pseudoalteromonas prydzensis*	KX417143	0.29	*Hyphomonas* sp.	KM2790018	0.17	*Marivita roseacus*	NR_132662	0.37	*Thalassospira* sp.	LN868390	0.10	*Oceanicaulis alexandrii*	FN562403	0.19
18	*Hoeflea* sp.	KC469076	0.28	*Halomonas* sp.	KX780067	0.15	Unclassified		0.33				*Marivita roseacus*	NR_132662	0.17
19	*Sphingomonas* sp.	KX419227	0.25	*Stappia* sp.	LT629784	0.10	*Stappia indica*	KY616246	0.33				*Marivita roseacus*	NR_132662	0.17
20	Unclassified		0.23	*Phyllobacteriaceae bacterium*	KM279375	0.12	*Hoeflea* sp.	KC469076	0.32				*Pseudomonas* sp.	KY020318	0.16

Table 4-1. Comparison of shared and specific OTUs on day 5.

Sample	No. of OTUs	Initial (day 0)		Red (5R)		Yellow (5Y)	
		OTUs (%)	Reads (%)	OTUs (%)	Reads (%)	OTUs (%)	Reads (%)
I-R-Y	26	26.26	86.10	27.66	62.57	26.53	76.57
I-R	10	10.10	1.50	10.64	1.20	-	-
I-Y	15	15.15	5.26	-	-	15.31	18.97
R-Y	18	-	-	19.15	1.25	18.37	2.26
I	48	48.48	7.14	-	-	-	-
R	40	-	-	42.55	34.98	-	-
Y	39	-	-	-	-	39.80	2.20
Total	196	100.00	100.00	100.00	100.00	100.00	100.00

Table 4-2. Comparison of shared and specific OTUs on day 15.

Sample	No. of OTUs	Red15R		Red15Y	
		OTUs (%)	Reads (%)	OTUs (%)	Reads (%)
R-Y	37	47.44	97.31	38.54	91.20
R	41	52.56	2.69	-	-
Y	59	-	-	61.46	8.80
Total	137	100.00	100.00	100.00	100.00

Table 4-3. Comparison of the shared and specific OTUs of two separate samples grown under the red light.

Sample	No. of OTUs	Red1		Red2	
		OTUs (%)	Reads (%)	OTUs (%)	Reads (%)
R1-R2	53	55.21	98.56	56.38	97.93
R1	43	44.79	1.44	-	-
R2	41	-	-	43.62	2.07
Total	137	100.00	100.00	100.00	100.00

Table 4-4. Comparison of the shared and specific OTUs of two separate samples grown under the yellow light.

Sample	No. of OTUs	Yellow 1		Yellow 2	
		OTUs (%)	Reads (%)	OTU (%)	Reads (%)
Y1-Y2	13	13.27	62.73	13.13	3.89
Y1	85	86.73	37.27	-	-
Y2	86	-	-	86.87	96.11
Total	184	100.00	100.00	100.00	100.00

cultures were compared (Fig. 4). Photosynthesis occurred well in the red wavelength, and the microbial community was rapidly transformed so that the community structure was similar on day 5. On the other hand, the yellow wavelength had relatively slow photosynthesis, and the bacteria showed more influence on each other. The number of OTUs shared within the two cultures was small even under the same condition due to the randomly inoculated bacteria, as expected (Table 4-4 and Figs. 3C and 4).

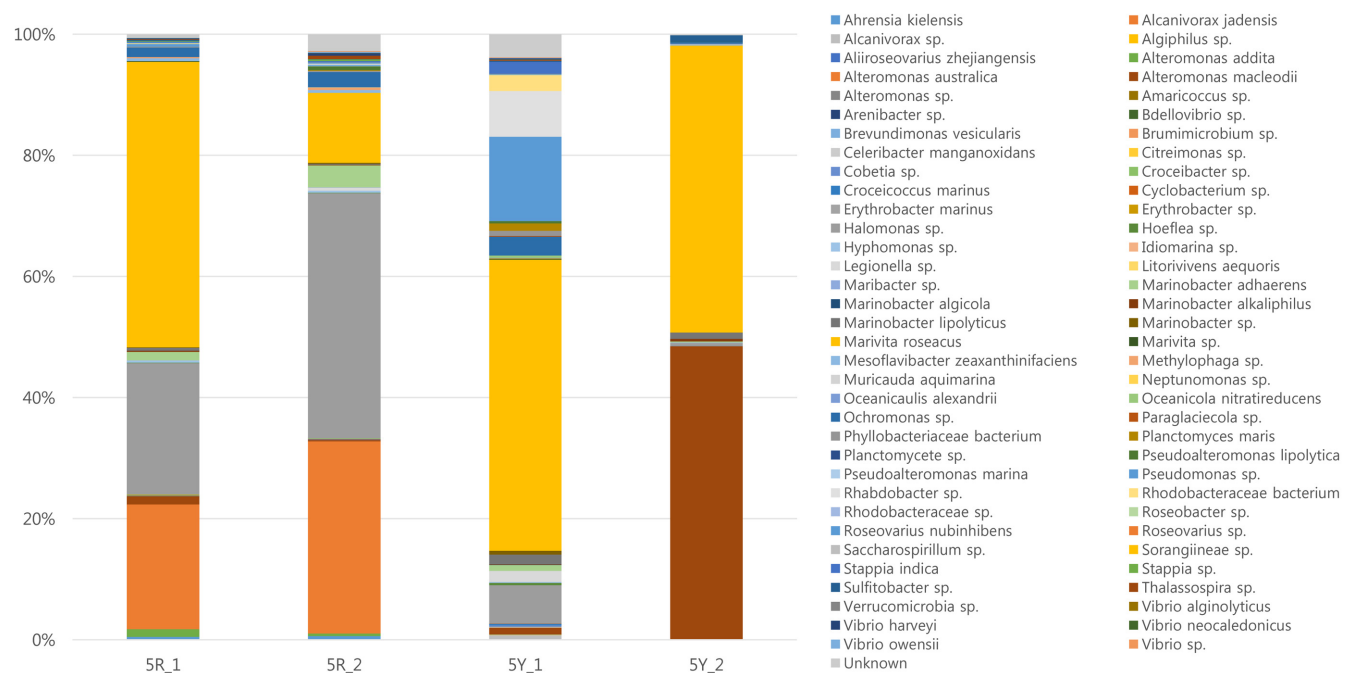As a result, the red wavelength has a positive effect on

Ahrensia kielensis
Alcanivorax sp.
Aliiroseovarius zhejiangensis
Alteromonas australica
Alteromonas sp.
Arenibacter sp.
Brevundimonas vesicularis
Celeribacter manganoxidans
Cobetia sp.
Croceicoccus marinus
Erythrobacter marinus
Halomonas sp.
Hyphomonas sp.
Legionella sp.
Maribacter sp.
Marinobacter algicola
Marinobacter lipolyticus
Marivita roseacus
Mesoflavibacter zeaxanthinifaciens
Muricauda aquimarina
Oceanicaulis alexandrii
Ochromonas sp.
Phyllobacteriaceae bacterium
Planctomycete sp.
Pseudoalteromonas marina
Rhabdobacter sp.
Rhodobacteraceae sp.
Roseovarius nubinhibens
Saccharospirillum sp.
Stappia indica
Sulfitobacter sp.
Verrucomicrobia sp.
Vibrio harveyi
Vibrio owensii
Unknown

Alcanivorax jadensis
Algiphilus sp.
Alteromonas addita
Alteromonas macleodii
Amaricoccus sp.
Bdellovibrio sp.
Brumimicrobium sp.
Citreimonas sp.
Croceibacter sp.
Cyclobacterium sp.
Erythrobacter sp.
Hoeflea sp.
Idiomarina sp.
Litorivivens aequoris
Marinobacter adhaerens
Marinobacter alkaliphilus
Marinobacter sp.
Marivita sp.
Methylophaga sp.
Neptunomonas sp.
Oceanicola nitratireducens
Paraglaciecola sp.
Planctomyces maris
Pseudoalteromonas lipolytica
Pseudomonas sp.
Rhodobacteraceae bacterium
Roseobacter sp.
Roseovarius sp.
Sorangiineae sp.
Stappia sp.
Thalassospira sp.
Vibrio alginolyticus
Vibrio neocaledonicus
Vibrio sp.

Fig. 4. Bacterial community structures at the species level on day 5 of the red and yellow wavelengths.

Table 5. Microbial species observed on day 15 samples under the red and yellow LED lights.

	Day 15 - Red						Day 15 - Yellow				
Species	Contigs (%)	Phylum	Class	Order	Family	Species	Contigs (%)	Phylum	Class	Order	Family
Marivita roseacus	88.64	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Marivita roseacus*	88.12	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae
Halomonas sp.	4.92	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Rhabdobacter* sp.	4.2	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae
Rhabdobacter sp.	2.09	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*Legionella* sp.	2.16	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae
Oceanicaulis alexandrii	1.28	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hypomonadaceae	*Planctomyces maris*	1.29	Proteobacteria	Planctomycetia	Planctomycetales	Planctomycetaceae
Phyllobacteriaceae bacterium	0.77	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Thalassospira* sp.	0.63	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae
Methylophaga sp.	0.36	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	*Oceanicaulis alexandrii*	0.5	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hypomonadaceae
Legionella sp.	0.35	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	*Planctomycete* sp.	0.39	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae
Marinobacter adhaerens	0.35	Proteobacteria	Gammaproteobacteria	Alteromanadales	Alteromonadaceae	*Halomonas* sp.	0.33	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae
Thalassospira sp.	0.25	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Phyllobacteriaceae bacterium*	0.3	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae
Planctomyces maris	0.23	Proteobacteria	Planctomycetacia	Planctomycetales	Planctomycetaceae	*Methylophaga* sp.	0.3	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae
Hyphomonas sp.	0.11	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	*Pseudoalteromonas lipolytica*	0.28	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudanabaenaceae
Pseudoalteromonas lipolytica	0.11	Proteobacteria	Gammaproteobacteria	Alteromanadales	Psedonabaenaceae	*Brunimicrobium* sp.	0.26	Bacteroidetes	Flavobacteria	Flavobacteriales	Cryomorphaceae
Rhodobacteriaceae bacterium	0.08	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Sorangiinae* sp.	0.25	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae (Labilithrix)
Brunimicrobium sp.	0.07	Bacteroidetes	Flavobacteria	Flavobacteriales	Cryomorphaceae	*Rhodobacteriaceae bacterium*	0.24	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae
Marinobacter lipolyticus	0.07	Proteobacteria	Gammaproteobacteria	Alteromanadales	Alteromonadaceae	*Marinobacter lipolyticus*	0.23	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae
Alcanivorax jadensis	0.06	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Alcanivoracaceae	*Hoeflea* sp.	0.11	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae
Roseovarius nubinhibens	0.06	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Marivita* sp.	0.1	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae
Hoeflea sp.	0.06	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Marinobacter adhaerens*	0.07	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae
Litoriivivens aequoris	0.06	Proteobacteria	Gammaproteobacteria	Unclassified	Unclassified	*Alcanivorax jadensis*	0.06	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Alcanivoracaceae
Sorangiinae sp.	0.03	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae (Labilithrix)	*Hyphomonas* sp.	0.06	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae
Marivita sp.	0.02	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Litoriivivens aequoris*	0.05	Proteobacteria	Gammaproteobacteria	Unclassified	Unclassified
Planctomycete sp.	0.02	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	*Roseovarius nubinhibens*	0.03	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae
Sulfitobacter sp.	0.02	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Sulfitobacter* sp.	0.03	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae

the growth of *T. suecica*. However, its growth was also affected by the randomly established initial bacterial community structure, which may be related to the difference in the metabolites the bacteria release. As photosynthetic microalgae growth increases in 5−10 days after inoculation, consuming the carbon dioxide produced by the heterotrophic bacteria, the initial microbial community continues to change, optimizing themselves in the oxygen-rich environment. In fact, aerobic bacteria can promote microalgal growth by reducing the photosynthetic oxygen in nature [32]. We could therefore expect that the remaining species would be aerobic heterotrophic or photosynthetic bacteria (Table 5). On day 15, all remaining species in the red and yellow lights were aerobic bacteria due to oxygen produced by the photosynthesis of *T. suecica*. Especially, *M. roseacus* seems to be the most dominant because it is aerobic and can photosynthesize. This study can be taken further by adding organic matter or specific bacteria and more algae. Actually, earlier studies proposed that amino acids and vitamins regulate the relationship between microalgae and bacteria [33, 34]. When cultured in medium with extra additives, beneficial effects were enhanced extending our knowledge of interaction between the microalgae and its associated bacteria [12].

In conclusion, we herein introduced the NGS technique to analyze the precise microbial community structure changes during the culture of *T. suecica*, which has been difficult and complicated to accomplish by conventional methods. As a result, we were able to extend our knowledge about the interaction between microalgae and heterotrophic bacteria in a complicated aquatic microbial ecosystem. Algae-bacteria consortia can be seen as a very important concept in understanding the aquatic environment and ecosystem, and may also provide important knowledge for biotechnological applications including in wastewater treatment, bioremediation, and sustainable aquaculture [35].

Acknowledgment

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Muller-Feuga A, Robert R, Cahu C, Robin J, Divanach P. 2003. Uses of microalgae in aquaculture. *Live Feeds Marine Aquac.* 253-299.

2. Austin B, Day JG. 1990. Inhibition of prawn pathogenic *Vibrio* spp. by a commercial spray-dried preparation of *Tetraselmis suecica*. *Aquaculture* **90:** 389-392.

3. Austin B, Baudet E, Stobie M. 1992. Inhibition of bacterial fish pathogens by *Tetraselmis suecica*. *J. Fish Dis.* **15:** 55-61.

4. Irianto A, Austin B. 2002. Probiotics in aquaculture. *J. Fish Dis.* **25:** 633-642.

5. Carballo-Cárdenas EC, Tuan PM, Janssen M, Wijffels RH. 2003. Vitamin E (α-tocopherol) production by the marine microalgae *Dunaliella tertiolecta* and *Tetraselmis suecica* in batch cultivation. *Biomol. Eng.* **20:** 139-147.

6. Han MA, Hong SJ, Kim ZH, Cho BK, Lee H, Choi HK, *et al*. 2018. Enhanced production of fatty acids via redirection of carbon flux in marine microalga *Tetraselmis* sp. *J. Microbiol. Biotechnol.* **28:** 267-274.

7. Lee SJ, Choi WS, Park GH, Kim TH, Oh C, Heo SJ, *et al*. 2018. Flocculation Effect of Alkaline Electrolyzed Water (AEW) on Harvesting of Marine Microalga *Tetraselmis* sp. *J. Microbiol. Biotechnol.* **28:** 432-438.

8. Materassi R, Tredici M, Milicia F, Sili C, Pelosi E, Vincenzini M, *et al.* 1983. Development of a production size system for the mass culture of marine microalgae. *Energy from Biomass.* pp. 150-158*. Reidel Publishing Company, Boston.*

9. Fabregas J, Herrero C, Cabezas B, Abalde J. 1985. Mass culture and biochemical variability of the marine microalga *Tetraselmis suecica* Kylin (Butch) with high nutrient concentrations. *Aquaculture* **49:** 231-244.

10. Abiusi F, Sampietro G, Marturano G, Biondi N, Rodolfi L, D'Ottavio M, *et al.* 2014. Growth, photosynthetic efficiency, and biochemical composition of *Tetraselmis suecica* F&M-M33 grown with LEDs of different colors. *Biotechnol. Bioeng.* **111:** 956-964.

11. Zhao Y, Wang J, Zhang H, Yan C, Zhang Y. 2013. Effects of various LED light wavelengths and intensities on microalgae-based simultaneous biogas upgrading and digestate nutrient reduction process. *Bioresour. Technol.* **136:** 461-468.

12. Han J, Zhang L, Wang S, Yang G, Zhao L, Pan K. 2016. Co-culturing bacteria and microalgae in organic carbon containing medium. *J. Biol. Res.-Thessaloniki.* **23:** 8.

13. Ramanan R, Kim BH, Cho DH, Oh HM, Kim HS. 2016. Algae-bacteria interactions: evolution, ecology and emerging applications. *Biotechnol. Adv.* **34:** 14-29.

14. Biondi N, Cheloni G, Tatti E, Decorosi F, Rodolfi L, Giovannetti L, *et al.* 2017. The bacterial community associated with *Tetraselmis suecica* outdoor mass cultures. *J. Appl. Phycol.* **29:** 67-78.

15. Fierer N, Jackson JA, Vilgalys R, Jackson RB. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl. Environ. Microbiol.* **71:** 4117-4120.

16. Zhao Y, Duan C, Zhang X, Chen H, Ren H, Yin Y, Ye L. 2018. Insights into the gut microbiota of freshwater shrimp and its associations with the surrounding microbiota and environmental factors. *J. Microbiol. Biotechnol.* **28:** 946-956.

17. Chen Y, Ye L, Zhao F, Xiao L, Cheng S, Zhang XX. 2017. Bacterial community shift during the startup of a full-scale oxidation ditch treating sewage. *J. Microbiol. Biotechnol.* **27:** 141-148.

18. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* **79:** 5112-5120.

19. Kim TH, Lee Y, Han SH, Hwang SJ. 2013. The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using *Scenedesmus* sp. for wastewater treatment. *Bioresour. Technol.* **130:** 75-80.

20. Oh SJ, Kwon HK, Jeon JY, Yang HS. 2015. Effect of monochromatic light emitting diode on the growth of four microalgae species (*Chlorella vulgaris*, *Nitzschia* sp., *Phaeodactylum tricornutum*, *Skeletonema* sp.). *J. Kor. Soc. Mar. Env. Saf.* **21:** 1-8.

21. Medler S, Mykles DL. 2003. Analysis of myofibrillar proteins and transcripts in adult skeletal muscles of the American lobster *Homarus americanus*: variable expression of myosins, actin and troponins in fast, slow-twitch and slow-tonic fibres. *J. Exp. Biol.* **206:** 3557-3567.

22. Yu X, Mykles DL. 2003. Cloning of a muscle-specific calpain from the American lobster *Homarus americanus*: expression associated with muscle atrophy and restoration during moulting. *J. Exp. Biol.* **206:** 561-575.

23. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, *et al.* 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75:** 7537-7541.

24. Staley C, Unno T, Gould T, Jarvis B, Phillips J, Cotner J, *et al.* 2013. Application of Illumina next-generation sequencing to characterize the bacterial community of the Upper Mississippi River. *J. Appl. Microbiol.* **115:** 1147-1158.

25. Thijs S, Op De Beeck M, Beckers B, Truyens S, Stevens V, Van Hamme JD, *et al.* 2017. Comparative evaluation of four bacteria-specific primer pairs for 16S rRNA gene surveys. *Front Microbiol.* **8:** 494.

26. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, *et al.* 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **41:** e1. doi: 10.1093/nar/gks808

27. Ugwu C, Aoyagi H, Uchiyama H. 2007. Influence of irradiance, dissolved oxygen concentration, and temperature on the growth of *Chlorella sorokiniana*. *Photosynthetica.* **45:** 309-311.

28. Makridis P, Ferreira T, Kokou F, Tsigenopoulos CS, Divanach P. 2012. Quantitative and qualitative aspects of bacterial communities associated with cultures of *Chlorella minutissima*. *J. World Aquacul. Soc.* **43:** 571-578.

29. Lee JK, Lim JH, Lee TY. 2012. Cultivation of *Tetraselmis suecica* under different types of light emitting diodes. *Int. J. Environ.* **21:** 757-761.

30. Green DH, Echavarri-Bravo V, Brennan D, Hart MC. 2015. Bacterial diversity associated with the coccolithophorid algae *Emiliania huxleyi* and *Coccolithus pelagicus* f. *braarudii*. *Biomed. Res. Int.* **2015:** 194540.

31. Budinoff CR, Dunlap JR, Hadden M, Buchan A. 2011. *Marivita roseacus* sp. nov., of the family Rhodobacteraceae, isolated from a temperate estuary and an emended description of the genus Marivita. *J. Gen. Appl. Microbiol.* **57:** 259-267.

32. Mouget JL, Dakhama A, Lavoie MC, de la Noüe J. 1995. Algal growth enhancement by bacteria: Is consumption of photosynthetic oxygen involved? *FEMS Microbiol. Ecol.* **18:** 35-43.

33. Ohwada K. 1973. Seasonal cycles of vitamin B12, thiamine and biotin in Lake Sagami. Patterns of their distribution and ecological significance. *Int. Rev. Hydrobiol.* **58:** 851-871.

34. Ohwada K, Taga N. 1972. Distribution and seasonal variation of vitamin B12, thiamine and biotin in the sea. *Mar. Chem.* **1:** 61-73.

35. Fuentes JL, Garbayo I, Cuaresma M, Montero Z, González-del-Valle M, Vílchez C. 2016. Impact of microalgae-bacteria interactions on the production of algal biomass and associated compounds. *Mar. Drugs.* **14**(5). pii: E100.