Research Article

Meixia Pang#, Zhili Huang#, Le Lv, Xiaodong Li, Gang Jin*

Seasonal succession of bacterial communities in cultured Caulerpa lentillifera detected by high-throughput sequencing

https://doi.org/10.1515/biol-2022-0001
received March 27, 2021; accepted October 19, 2021

Abstract: An increasing number of microorganisms are being identified as pathogens for diseases in macroalgae, but the species composition of bacteria related to Caulerpa lentillifera, fresh edible green macroalgae worldwide, remains largely unclear. The bacterial communities associated with C. lentillifera were investigated by high-throughput 16S rDNA sequencing, and the bacterial diversities in washed and control groups were compared in this study. A total of 4,388 operational taxonomic units were obtained from all the samples, and the predominant prokaryotic phyla were Proteobacteria, Bacteroidetes, Planctomycetes, Cyanobacteria, Actinobacteria, Verrucomicrobia, Chloroflexi, and Acidobacteria in C. lentillifera. The bacterial diversity changed with seasons and showed an increasing trend of diversity with the rising temperature in C. lentillifera. There were slight reductions in the abundance and diversity of bacteria after washing with tap water for 2 h, indicating that only parts of the bacterial groups could be washed out, and hidden dangers in C. lentillifera still exist. Although the reduction in the abundance of some bacteria revealed a positive significance of washing C. lentillifera with tap water on food safety, more effective cleaning methods still need to be explored.

Keywords: 16S rDNA, bacterial diversity, Caulerpa lentillifera, washing with tap water

1 Introduction

The genus Caulerpa (Bryopsidales, Chlorophyta), a siphonous green macroalga, is widely distributed in subtropics and tropics, such as China, Singapore, Indonesia, the Philippines, Malaysia, Vietnam, and Japan. Some species of the genus are consumed as vegetables blended with onion, vinegar, or tomatoes [1]. For example, Indonesian Caulerpa usually is served as a side dish in South Sulawesi [2] and is used in fresh salads in Japan and many Asian regions [3]. C. lentillifera (sea grape), one of the most popular edible green macroalgae [1], which looks like grapes and is thus called green caviar [4–6].

The algae of genus Caulerpa are high in several vitamins and minerals, including iron, calcium, magnesium, and iodine [7–9]. Moreover, C. lentillifera contains a high level of polyunsaturated fatty acids and multiple essential amino acids (EAA) with low-level total lipid content [10]. The EAA composition of C. lentillifera approaches the ideal model recommended by the Food and Agriculture Organization/World Health Organization [11], and the reported protein content varies from 3.6 to 19.4% dry weight mass of C. lentillifera [6,12,13]. C. lentillifera also has potential functions, such as antidiabetic activity [14,15], anti-inflammatory activity [16], immunostimulatory activity [17], preventing hypertension [18], as well as anticoagulant and anticancer activity [19]. In recent years, C. lentillifera was introduced into China for a large-scale artificial cultivation as functional seafood. C. lentillifera was cultured with sand-filtered seawater on a double-layer net, under which a layer of sand was laid as an attachment base for rhizomes. The artificially cultivated C. lentillifera were raised in muddy ponds following standard culture conditions (20.0–32.0°C, 5,000–10,000 Lux) and were harvested regularly.

Diseases caused by seafood pose a critical hazard to public health worldwide [20]. The global consumption of seafood per capita has increased over the last few years [21]. The import and domestic aquaculture of seafood
have also increased. Besides, some recent human gastroenteritis outbreaks have been associated with contaminated seafood consumption [22]. More details on pathogen virulence and pathogenicity should be obtained to investigate the seafood-borne disease caused by pathogens such as norovirus and Vibrio [3]. There are many foodborne pathogens in the marine environments, which may attach to the surface of seafood and enter the human body on consumption in fresh and live forms, thereby leading to several health risks. For example, Vibrio parahaemolyticus is a facultative, anaerobic, gram-negative bacterium with a curved rod shape, usually found in an estuary or marine environment, and causes spoilage of C. lentillifera [3]. An increasing number of microbes are being identified as pathogens of macroalgal disease [23], but bacteria attached to C. lentillifera remain largely unclear for the species composition. In addition, the consumption of the cultured C. lentillifera directly after washing with tap water is quite common [24]. Our study aims to identify bacterial communities associated with C. lentillifera by high-throughput 16S rDNA sequencing and explore whether washing with tap water can eliminate some pathogenic bacteria. Our results regarding the bacterial characteristics illustrated the structure of C. lentillifera microflora and determined the effectiveness of washing for food safety of C. lentillifera.

2 Materials and methods

2.1 Sample collection and DNA extraction

C. lentillifera samples, cultured with sand-filtered seawater pumped from the South China Sea, were collected from culture ponds in Shenzhen, Guangdong province of China (114°03′ E/22°44′ N). C. lentillifera materials were collected monthly from June 2018 to May 2019, except in January as the species was absent. Then, C. lentillifera materials collected each time were assigned to the control group (marked as S) and washed group (marked as SW). For the washed group, C. lentillifera materials were soaked with chlorinated tap water for 2 h, and the water was changed four times for 30 min each during the washing process. All the samples were stored at −80.0°C until further processing. C. lentillifera samples were named the group marker plus the collection time. For example, S1806 was collected in June 2018 and divided into the control group; and SW1905 was collected in May 2019 and divided into the washed group (Table 1).

The sampling temperature of seawater was 20.0–29.5°C with an average temperature of 25.0°C. When dividing the sampling time into four seasons, the average temperatures in summer (labeled as 1), autumn (labeled as 2), winter (labeled as 3), and spring (labeled as 4) were 29.0, 24.0, 20.25, and 25.2°C, respectively. To understand the diversities and variability of microorganisms with the change of seasons in C. lentillifera, all the samples were assigned to seasonal subgroups. Detailed information is shown in Table 1. All samples were collected monthly. Three replicates per season were performed.

For DNA extraction from C. lentillifera, TIANamp Stool DNA Kit (Tiangen, Beijing, China) was used according to the manufacturer’s protocol. The obtained DNA integrity was tested by 1% agarose gel electrophoresis and quantified using the PicoGreen dsDNA quantitation assay (Invitrogen, Carlsbad, CA), and the extracts were stored at −20.0°C.

2.2 16S rDNA library generation and microbiome sequencing

The universal primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′), together with 806R (5′-GGACTACHVGGGTWTCTAAT-3′), were used to amplify the bacterial 16S rRNA gene V4 hypervariable region of DNA samples following specific procedures. The polymerase chain reaction (PCR) reaction system consisted of 1× Hi-Fidelity buffer, 30 ng qualified genomic DNA, dNTP PurePeak DNA polymerase mix (200 µM, Pierce Nucleic Acid Technologies, Milwaukee, WI, USA), Platinum Taq High Fidelity Polymerase (1 unit, Life Technologies, Carlsbad, CA, USA), MgCl2 (2.0 mM, 0.06% BSA, along with forward and reverse primers (0.2 µM each). PCR amplification parameters were set as follows: 3 min of initial denaturation under 98.0°C; 45 s under 98.0°C, 45 s under 55.0°C, and 45 s under 72.0°C for 30 cycles; 7 min of extension under 72.0°C. Then, the Agencourt AMPure XP magnetic beads were utilized to purify the amplified PCR products, eventually dissolved into the elution buffer. The Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) was adopted to test the DNA libraries, while the HiSeq platform was used for pair-end sequencing, with the PE250 sequencing strategy was used (PE251 + 8 + 8 + 251; HiSeq SBS Kit V2, Illumina) under specific protocols.

2.3 Sequence analysis and bioinformatics

Clean data were obtained by filtering low-quality sequences from Raw fastq files using the program Quantitative
Insights Into Microbial Ecology (version 1.9.1) (http://www.wernerlab.org/software/macqiime) [25], as described in an earlier study [26]. Then, Fast Length Adjustment of Short reads software (v1.2.11) [27] was used to merge those pair-end reads for obtaining tags that contained the V4 hypervariable region, the minimal matching length was 15 bp, whereas the mismatch ratio was 0.1 within the overlapped regions. UPARSE [28] was utilized to cluster the operational taxonomic units (OTUs) with a similarity threshold of 97%, whereas UCHIME [29] was used to identify and remove the chimeric sequences. The sequence NCBI number was PRJNA658212. For assigning OTUs to the nearest matching described taxon, the Greengenes taxonomy database (version 13_5) was adopted to query sequences for 16S rRNA genes [30]. Finally, each quality-filtered read was mapped by the usearch_global algorithm to the eventual set, which represented OTU sequences [31], to obtain the community composition of each sample.

In the samples, the microorganism alpha-diversity indices were evaluated according to the annotated data, including the observed species index, Chao I richness, ace index, Shannon index, and good coverage [32]. Among them, the observed species, Chao I richness, and ace index reflected the species richness of the bacterial community. The rarecurve function was used to calculate and plot the rarefaction curves [33], corresponding to the observed species in the R package vegan. Shannon index presents the diversity of microbial species and can be impacted by species evenness and richness of a sample community, while good coverage is a value representing sequencing coverage of the sample library. The relative abundance (RA) of the bacterial community composition of the samples was evaluated at the levels of phylum, class, and genus. Multiple comparisons of the bacterial alpha-diversity indices and RA between the different groups (or subgroups) were subject to one-way analysis of variance and Tukey’s HSD post hoc test using the SPSS 19.0 software. The results were presented as mean ± standard error, and differences were considered significant at p < 0.05.

Linear discriminant analysis effect size (LEfSe) [34] has been developed as an approach to discover and explain biomarkers for high-dimensional data. In LEfSe, statistical significance is applied in combination with the estimation of effective size and biological consistency. In this study, LEfSe was adopted to discover biomarkers based on microorganisms. In contrast, LEfSe analysis-derived linear discriminant analysis (LDA) scores were adopted for displaying the association across taxa by the cladogram

Table 1: Detail information about all samples used in this study

| Samples | Group name | Subgroup name | Tag number | OTU number | Sample time | Water temperature (°C) |
|---------|------------|---------------|------------|------------|-------------|------------------------|
| S1806   | S          | S1            | 33,742     | 1,133      | June 2018   | 28.5                   |
| S1807   | S          | S1            | 34,397     | 1,733      | July 2018   | 29.5                   |
| S1808   | S          | S1            | 40,463     | 1,433      | August 2018 | 29.0                   |
| S1809   | S          | S2            | 42,789     | 1,223      | September 2018 | 26.0               |
| S1810   | S          | S2            | 31,559     | 1,141      | October 2018 | 24.5                   |
| S1811   | S          | S2            | 40,601     | 1,222      | November 2018 | 21.5                  |
| S1812   | S          | S3            | 41,679     | 1,160      | December 2018 | 20.5                  |
| S1902   | S          | S3            | 40,713     | 1,129      | February 2019 | 20.0                  |
| S1903   | S          | S4            | 42,186     | 719        | March 2019   | 23.0                   |
| S1904   | S          | S4            | 40,097     | 1,696      | April 2019   | 25.5                   |
| S1905   | S          | S4            | 40,377     | 1,358      | May 2019     | 27.0                   |
| SW1806  | SW         | SW1           | 35,689     | 1,071      | June 2018   | 28.5                   |
| SW1807  | SW         | SW1           | 31,833     | 1,341      | July 2018   | 29.5                   |
| SW1808  | SW         | SW1           | 41,677     | 1,260      | August 2018 | 29.0                   |
| SW1809  | SW         | SW2           | 42,573     | 1,259      | September 2018 | 26.0               |
| SW1810  | SW         | SW2           | 33,145     | 1,363      | October 2018 | 24.5                   |
| SW1811  | SW         | SW2           | 42,496     | 510        | November 2018 | 21.5                  |
| SW1812  | SW         | SW3           | 35,814     | 1,474      | December 2018 | 20.5                  |
| SW1902  | SW         | SW3           | 45,465     | 701        | February 2019 | 20.0                  |
| SW1903  | SW         | SW4           | 42,498     | 820        | March 2019   | 23.0                   |
| SW1904  | SW         | SW4           | 40,855     | 1,507      | April 2019   | 25.5                   |
| SW1905  | SW         | SW4           | 43,602     | 592        | May 2019     | 27.0                   |

S: control group and SW: washed group. S1, S2, S3, and S4: control C. lentillifera samples collected in summer, autumn, winter, and spring; and SW1, SW2, SW3, and SW4: washed C. lentillifera samples collected in summer, autumn, winter, and spring. All samples were collected monthly. Three replicates per season were performed.
(circular hierarchical tree) regarding those remarkably up-regulated and downregulated microbial taxa between two groups. In each sample, the biomarker taxon RA was presented in straight dotted lines, and the medians and averages for subgroups were also plotted. The levels of the branch graph represent the phylum, class, order, family, and genus from the inner to the outer circles. The color codes and the letters indicate the groups and the taxa, respectively, that contribute to the uniqueness of the corresponding groups when LDA > 2.0.

3 Results

3.1 Richness and diversity

In this study, we first analyzed the overall microbial diversity in a total of 22 *C. lentillifera* samples across four seasons. The samples were sequenced, yielding 0.86 million short-read V4 16S rRNA gene sequences (31,559–45,465 per library; Table 1). After strict quality and size filtering, high-quality sequences were clustered into 4,388 OTUs corresponding to the bacterial community (510–1,733 per sample; Table 1). Observed species rarefaction curves reached coverages of more than 0.95 (Table 2), suggesting that a very reasonable sequencing depth has been attained (Figure 1).

Among all the detected OTUs, 2,946 OTUs were shared in the S and SW groups, whereas 616 and 233 OTUs were specific in the S and SW groups, respectively (Figure S1). A total of 3,562 OTUs were found in the S group, of which 919 OTUs were shared in all seasons, whereas 411, 205, 136, and 341 OTUs were specific in summer, autumn, winter, and spring, respectively (Figure S2). Most OTUs were found in summer, followed by spring, autumn, and winter.

Furthermore, a total of 3,179 OTUs was found in the SW group, with 871 OTUs shared among all the seasons, and 387, 226, 171, and 253 OTUs specific in the summer, autumn, winter, and spring, respectively, and a general lower RA than in the S group (Figure S3).

The average number of observed species, the community richness, and the community diversity in each group are shown in Table 2. As for the bacterial diversity of the S group, the average values of observed species, Chao1, ace index, and Shannon index were 923.9 ± 214.8, 1308.1 ± 272.3, 1440.8 ± 297.8, and 3.7 ± 0.9, respectively. The highest values of the richness indices and diversity indices appeared in summer, and then they decreased as the temperature decreased and, finally, increased with the arrival of spring and the rise in the temperature

![Figure 1: Observed species rarefaction curves of all samples. S: control group and SW: washed group.](image)

Table 2: Average alpha-diversity indices of the different group samples

| Group | Subgroup | Observed species | Chao1 richness | Ace index | Shannon index | Good coverage |
|-------|----------|------------------|----------------|-----------|---------------|---------------|
| S     | S1       | 1062.5 ± 239.6<sup>a</sup> | 1453.3 ± 273.2<sup>a</sup> | 1555.8 ± 257.5<sup>a</sup> | 4.8 ± 0.6<sup>a</sup> | 0.950 ± 0.009<sup>a</sup> |
|       | S2       | 867.2 ± 60.2<sup>a</sup> | 1243.1 ± 20.4<sup>a</sup> | 1365.4 ± 88.2<sup>a</sup> | 3.1 ± 0.4<sup>a</sup> | 0.958 ± 0.006<sup>a</sup> |
|       | S3       | 820.8 ± 17.3<sup>a</sup> | 1213.1 ± 86.8<sup>a</sup> | 1370.4 ± 206.1<sup>a</sup> | 3.0 ± 0.5<sup>b</sup> | 0.962 ± 0.000<sup>a</sup> |
|       | S4       | 910.7 ± 353.6<sup>a</sup> | 1291.3 ± 494.3<sup>a</sup> | 1448.2 ± 560.8<sup>a</sup> | 3.5 ± 0.7<sup>ab</sup> | 0.959 ± 0.013<sup>a</sup> |
|       | Total    | 923.9 ± 214.8    | 1308.1 ± 272.3 | 1440.8 ± 297.8 | 3.7 ± 0.9 | 0.957 ± 0.009 |
| SW    | SW1      | 889.8 ± 82.0<sup>a</sup> | 1274.3 ± 162.9<sup>a</sup> | 1399.4 ± 201.5<sup>a</sup> | 4.2 ± 0.5<sup>a</sup> | 0.955 ± 0.008<sup>a</sup> |
|       | SW2      | 759.2 ± 349.1<sup>a</sup> | 1104.3 ± 451.8<sup>a</sup> | 1215.7 ± 455.7<sup>a</sup> | 3.6 ± 1.4<sup>a</sup> | 0.962 ± 0.014<sup>a</sup> |
|       | SW3      | 796.8 ± 431.9<sup>a</sup> | 1116.3 ± 517.9<sup>a</sup> | 1241.2 ± 442.3<sup>a</sup> | 3.6 ± 2.1<sup>a</sup> | 0.962 ± 0.018<sup>a</sup> |
|       | SW4      | 685.4 ± 350.3<sup>a</sup> | 1045.7 ± 450.6<sup>a</sup> | 1269.5 ± 425.5<sup>a</sup> | 2.9 ± 1.1<sup>a</sup> | 0.967 ± 0.012<sup>a</sup> |
|       | Total    | 781.5 ± 274.6    | 1136.9 ± 349.5 | 1285.1 ± 333.6 | 3.6 ± 1.2 | 0.961 ± 0.012 |

S: control group and SW: washed group. S1, S2, S3, and S4: control *C. lentillifera* samples collected in summer, autumn, winter, and spring; and SW1, SW2, SW3, and SW4: washed *C. lentillifera* samples collected in summer, autumn, winter, and spring. The results were presented as mean ± standard error, and different letters indicate that there are significant differences among different groups (seasons) (*p* < 0.05).
In particular, the Shannon index values in autumn and winter were significantly lower than that in summer (S1_vs_S2, \( p = 0.027 \); S1_vs_S3, \( p = 0.036 \); Figure S4b). In the samples of the SW group, the average value of observed species, Chao1, ace index, and Shannon index were 781.5 ± 274.6, 1136.9 ± 349.5, 1285.1 ± 333.6, and 3.6 ± 1.2, respectively. However, the diversity decreased compared to that in the S group, with no significant difference between the two groups (\( p > 0.05 \), Table 2). The bacterial diversity of the SW group showed a seasonal trend similar to that observed in the S group. The richness and diversity indices appeared high in the summer and decreased in autumn and winter, with the lowest values in the spring. No significant differences were observed among the seasons (Figure S4c and d). As for the bacterial diversity of the S and SW groups in the same season, the richness and diversity indices in the SW group showed no significant difference to that observed in the S groups (\( p > 0.05 \), Figure S5).

### 3.2 Prokaryotic community composition and relationships based on phylum, class, and genus levels in different groups

Forty-three prokaryotic phyla were detected in all the samples, wherein 41 and 39 were detected in the S and SW groups, respectively. The predominant phyla observed were Proteobacteria (68.96%), Bacteroidetes (9.20%), Planctomycetes (8.13%), Cyanobacteria (5.57%), Actinobacteria (2.72%), Chloroflexi (2.04%), Acidobacteria (0.73%), and Verrucomicrobia (0.59%), which accounted for more than 98% of all the sequences. The unclassified prokaryotic phyla constituted 0.59% of all the sequences. The unclassified prokaryote at the genus level accounted for 85.53% of all the sequences (Table S1). The RA of prokaryotes at the Class level in different groups were associated with the SW group, accounting for 5.51, 3.84, and 3.55%, respectively (Table S1 and Figure 2b). The dominant bacterial communities in the SW group also included Planctomycetia (Planctomycetes), Sapropirae (Spirochaetes), and Flavobacteriia (Bacteroidetes), accounting for 5.51, 3.84, and 3.55%, respectively (Table S1 and Figure 2b). The dominant bacterial communities in the SW group also included Planctomycetia, Synechococyclaceae (Cyanobacteria), and Acidimicrobiia (Actinobacteria), accounting for 7.41, 5.27, and 3.03%, respectively (Table S1 and Figure 2b).

Only five prokaryotic genera were detected in all the samples in addition to others (6.89%), and the unclassified prokaryote at the genus level accounted for 85.53% of all the sequences (Table S1). The RA of prokaryotes at the genus level in different groups is shown in Figure 2c. The S group was dominated by Planctomyces (Planctomycetia, Planctomycetes; 1.27%) and Lyngbya (Cyanobacteria, Cyanophyta; 1.25%), and the SW group was dominated by Labrenzia (Alphaproteobacteria, Proteobacteria; 8.11%) and Planctomyces (1.72%) apart from those unclassified ones (Table S1 and Figure 2c).

For better understanding, the relationship between the bacterial community and the diverse structures of those treated C. lentillifera, this study conducted LEfSe analysis for determining those high-dimensional biomarker bacterial taxa of S versus SW samples (Figure 3a). Then, the cladogram (Figure 3b) was constructed to display the associations among the biomarker taxa. The results revealed that at the phylum level, Bacteroidetes was a biomarker bacteria related to the S group. At the class level, Flavobacteriia, Deltaproteobacteria (Proteobacteria), and Gammaproteobacteria were associated with the S group, and Alphaproteobacteria was associated with the SW group. The high-dimensional biomarker genera, such as Owenweeksia (Flavobacteria, Bacteroidetes), Flavobacterium (Flavobacteria, Bacteroidetes), Thalassospira (Alphaproteobacteria, Proteobacteria), Marivita (Alphaproteobacteria, Proteobacteria), Ruegeria (Alphaproteobacteria, Proteobacteria), Haliangiaceae (Deltaproteobacteria, Proteobacteria), Plesioscytis (Deltaproteobacteria, Proteobacteria), Alteromonas (Gammaproteobacteria, Proteobacteria), Glacieola (Gammaproteobacteria, Proteobacteria), Congregibacter (Gammaproteobacteria, Proteobacteria), Hahella (Gammaproteobacteria, Proteobacteria), Enterovibrio (Gammaproteobacteria, Proteobacteria), and Vibrio (Gammaproteobacteria, Proteobacteria) were associated with S group, and genera Labrenzia and Acinetobacter (Gammaproteobacteria, Proteobacteria) were related to SW group (Figure 3). As mentioned above, only Labrenzia has a RA of more than 0.5% in the groups.
3.3 Changes in the bacterial communities in two groups across the seasons

The distribution of the dominant bacterial groups in the main phyla exhibited no changes across seasons for S and SW groups. *Proteobacteria, Bacteroidetes, Planctomycetes, Cyanobacteria, and Actinobacteria* were listed among the top five bacterial communities in different seasons (Table 3).

In the S group, the abundance of *Planctomycetes* was the highest in summer, which decreased significantly in the other three seasons (S1_vs_S2, \( p = 0.007 \); S1_vs_S3, \( p = 0.006 \); and S1_vs_S4, \( p = 0.004 \); Figure S6a). Other dominant bacterial communities showed no significant temporal differences in the S group (\( p > 0.05 \), Table 3). In addition, the abundance of dominant bacterial communities showed no significant difference among these four seasons in the SW group (\( p > 0.05 \), Table 3).

Figure 2: Bacterial distribution in the different groups: (a) evaluated at the phylum taxonomical level, (b) evaluated at the class taxonomical level, and (c) evaluated at the genus taxonomical level. S: control group and SW: washed group.
The top five ranked dominant bacterial groups belonging to the main classes exhibited slight changes in the S group. The most and the second most predominant bacterial communities observed in the S group for all the seasons were Alphaproteobacteria (35.4–70.4%) and Gammaproteobacteria (5.4–11.5%), respectively (Table 4). In addition, Planctomycetia (9.8%), Synechococcophycideae (5.2%), and Acidimicrobiia (4.9%) were the other three dominant bacterial classes observed in summer; Planctomycetia (4.4 and 3.8%, respectively), Saprospirae (3.7 and 4.4%, respectively), and Flavobacteriia (2.5 and 2.0%, respectively) were dominant bacterial classes in autumn and winter. In comparison, Flavobacteriia (6.1%), Saprospirae (4.2%), and Planctomycetia (4.0%) were dominant bacterial classes in spring (Table 4). Among these dominant bacterial classes in the S group, the abundance of Alphaproteobacteria was the lowest in summer, increased

![LEfSe analysis in S and SW groups](image)

**Figure 3**: LEfSe analysis in S and SW groups: (a) LDA scores (log 10) derived from LEfSe analysis, showing the biomarker taxa for S and SW and (b) cladogram generated from LEfSe analysis showing the relationship between taxon. S: control group and SW: washed group.

| Group | Subgroup | Proteobacteria | Bacteroidetes | Planctomycetes | Cyanobacteria | Actinobacteria |
|-------|----------|----------------|---------------|----------------|---------------|---------------|
| S     | S1       | 52.7 ± 10.7a   | 10.9 ± 2.9a   | 14.0 ± 3.5a   | 7.0 ± 10.6a   | 5.0 ± 3.7a   |
| S2    | 77.6 ± 6.6a | 8.8 ± 2.6a   | 5.9 ± 0.9b   | 2.8 ± 3.7a   | 1.3 ± 0.2a   |
| S3    | 77.6 ± 7.2a | 8.2 ± 1.9a   | 4.7 ± 1.1b   | 3.6 ± 2.0a   | 1.6 ± 0.3a   |
| S4    | 69.2 ± 11.8a | 13.6 ± 6.2a  | 5.1 ± 0.8b   | 6.1 ± 7.9a   | 1.7 ± 1.3a   |
| SW    | SW1      | 55.1 ± 13.0a  | 10.0 ± 3.0a   | 12.3 ± 6.9a  | 9.8 ± 15.5a  | 4.8 ± 4.5a   |
| SW2   | 67.9 ± 14.2a | 10.0 ± 6.6a  | 10.5 ± 4.8a  | 2.2 ± 2.1a   | 3.9 ± 1.1a   |
| SW3   | 71.9 ± 24.8a | 6.4 ± 6.0a   | 8.4 ± 5.9a   | 6.6 ± 8.3a   | 2.5 ± 0.6a   |
| SW4   | 75.7 ± 17.0a | 5.8 ± 5.0a   | 5.2 ± 3.2a   | 8.4 ± 13.3a  | 1.3 ± 1.2a   |

**Table 3**: The main abundant bacterial phyla associated with the S and SW across seasons.

S: control group and SW: washed group. S1, S2, S3, and S4: control *C. lentillifera* samples collected in summer, autumn, winter, and spring; and SW1, SW2, SW3, and SW4: washed *C. lentillifera* samples collected in summer, autumn, winter, and spring. The results were presented as mean ± standard error, and different letters indicate that there are significant differences among different groups (seasons) (*p* < 0.05).
4 Discussion

A macroalgal community contains bacteria, fungi, diatoms, protozoa, spores, and larvae of marine invertebrates [35]. Among these attaching organisms, many bacteria and fungi have been identified as pathogens of macroalgal diseases [23]. However, bacteria show high abundance in the primary colonizers [36], whereas fungi are relatively rare in the sea [37]. In this study, the seasonal time-series autocorrelation of bacterial diversity in *C. lentillifera* was conducted by high-throughput 16S rDNA sequencing and revealed that washing with tap water slightly alters the microbiome of *C. lentillifera*.

Overall, in the present study, 5 major genera and 14 major classes of bacteria were detected in 43 phyla in *C. lentillifera* (Table S1). *Proteobacteria* and *Bacteroidetes* constituted the most abundant bacterial phyla associated with *C. lentillifera*, which was consistent with the earlier studies on other seaweeds [38,39], such as *Laminaria saccharina* [39], *L. hyperborea* [40], *Ulva australis* [41], *C. racemosa* [42], *Cystoseira compressa* [43], and *Sargassum muticum* [44]. *Alphaproteobacteria* and *Gammaproteobacteria* were the most and the second most

| Group | Subgroup | Alphaproteobacteria | Gammaproteobacteria | Planctomycetia | Synechococcophycideae | Saprospirae | Acidimicrobiia | Flavobacteriia | Anaerolineae |
|-------|----------|----------------------|----------------------|---------------|-----------------------|-------------|---------------|---------------|-------------|
| S     | S1       | 3.3 ± 0.5a           | 3.3 ± 0.5a           | 3.3 ± 0.5a    | 3.3 ± 0.5a            | 3.3 ± 0.5a  | 3.3 ± 0.5a    | 3.3 ± 0.5a    | 3.3 ± 0.5a   |
|       | S2       | 3.3 ± 0.5a           | 3.3 ± 0.5a           | 3.3 ± 0.5a    | 3.3 ± 0.5a            | 3.3 ± 0.5a  | 3.3 ± 0.5a    | 3.3 ± 0.5a    | 3.3 ± 0.5a   |
|       | S3       | 3.3 ± 0.5a           | 3.3 ± 0.5a           | 3.3 ± 0.5a    | 3.3 ± 0.5a            | 3.3 ± 0.5a  | 3.3 ± 0.5a    | 3.3 ± 0.5a    | 3.3 ± 0.5a   |
|       | S4       | 3.3 ± 0.5a           | 3.3 ± 0.5a           | 3.3 ± 0.5a    | 3.3 ± 0.5a            | 3.3 ± 0.5a  | 3.3 ± 0.5a    | 3.3 ± 0.5a    | 3.3 ± 0.5a   |
| SW    | SW1      | 3.3 ± 0.5a           | 3.3 ± 0.5a           | 3.3 ± 0.5a    | 3.3 ± 0.5a            | 3.3 ± 0.5a  | 3.3 ± 0.5a    | 3.3 ± 0.5a    | 3.3 ± 0.5a   |
|       | SW2      | 3.3 ± 0.5a           | 3.3 ± 0.5a           | 3.3 ± 0.5a    | 3.3 ± 0.5a            | 3.3 ± 0.5a  | 3.3 ± 0.5a    | 3.3 ± 0.5a    | 3.3 ± 0.5a   |
|       | SW3      | 3.3 ± 0.5a           | 3.3 ± 0.5a           | 3.3 ± 0.5a    | 3.3 ± 0.5a            | 3.3 ± 0.5a  | 3.3 ± 0.5a    | 3.3 ± 0.5a    | 3.3 ± 0.5a   |

S: control group and SW: washed group. S1, S2, S3, and S4: control samples collected in summer, autumn, winter, and spring; and SW1, SW2, SW3, and SW4: washed *C. lentillifera* samples collected in summer, autumn, winter, and spring. The results were presented as mean ± standard error, and different letters indicate that there are significant differences among different groups (seasons) (p < 0.05).
predominant classes related to \textit{C. lentillifera}, accounting for an average of 57.89 and 8.58% of the reads in the S and SW groups, respectively. While at the genus level, only five main prokaryotic genera were detected in addition to others (6.89%), and the abundance of unclassified prokaryote reached to 85.53% (Table S1), which needs to be further studied.

Temporal variations of \textit{C. lentillifera}-related bacterial microbial taxonomic composition were measured. The highest diversity of the \textit{C. lentillifera} bacterial community was revealed in summer, followed by spring, autumn, and winter, respectively, according to OUT richness and alpha diversity (Table 2), indicating that the bacterial diversity increases with the temperature of seasons. However, no significant temporal differences were found in the bacterial structure in \textit{C. lentillifera}, which may be due to the relatively stable temperature of Shenzhen throughout the year. Shenzhen (113°46′–114°37′ E, 22°27′–22°52′ N), one of the coastal cities in the south of China and near Hong Kong, has a mild climate with an annual average temperature of 23.0°C. The sampling temperature of seawater in this study was 20.0–29.5°C with an average temperature of 25.0°C, with a small shift across seasons. In addition, slight changes of the dominant bacterial groups contributed most of the dissimilarity across the seasons in \textit{C. lentillifera}. The most pronounced temporal changes in the microbial community of \textit{C. lentillifera} were abundantly increased in \textit{Planctomycetes} in summer (Table 3), which occurred primarily due to the increase of \textit{Planctomycetia} (Table 4). \textit{Planctomycetes} have been recognized as capable of mineralizing organic matters into inorganic counterparts, which fulfills the nutritional demands of the macroalgae [45–47]. Meanwhile, macroalgae are rich in \textit{Planctomycetes} [47, 48], the RA of which varies greatly depending on seaweed species and seasons [35]. The summer increase of \textit{Planctomycetes} in the present study is congruent with the studies reported on \textit{L. hyperboreae} [40] and \textit{Sargassum muticum} [44].

To our knowledge, this is the first study to investigate whether washing with tap water alters the microbiome associated with \textit{C. lentillifera} using high-throughput 16S rRNA gene sequencing and modern multivariate data analyzing software programs. The LEfSe analysis and cladogram visualization (Figure 3) revealed few types of biomarker bacteria associated with washed \textit{C. lentillifera}, and the most representative one was \textit{Labrenzia}. The RA of the genus \textit{Labrenzia}, belonging to the family \textit{Rhodobacteraceae}, was 50 times higher in \textit{C. lentillifera} after washing. \textit{Labrenzia} is the aerobic anoxygenic phototrophic bacterium that can generate little bacteriochlorophyll [49]. The abundance of \textit{Labrenzia} was found to be higher in healthy \textit{C. lentillifera} as compared to the diseased samples, which may contribute to the photosynthesis of algae [50]. Because the genus \textit{Labrenzia} was difficult to elute by tap water, it resulted in an increased abundance in \textit{C. lentillifera} even after washing, suggesting that there might be a symbiotic relationship between \textit{C. lentillifera} and \textit{Labrenzia}.

In China, the consumption of \textit{C. lentillifera} directly after washing is quite common, which might lead to bacterial infection. More types of high-dimensional biomarkers bacteria at different levels were associated with the S group, such as phyllum of \textit{Bacteroidetes}, classes of \textit{Flavobacteriia}, \textit{Flavobacterium}, \textit{Deltaproteobacteria}, and \textit{Gammaproteobacteria}, and genera of \textit{Haliangiaceae}, \textit{Plesiocystis}, \textit{Alteromonas}, \textit{Glaciecola}, or \textit{Congregibacter} (Figure 3). The abundance of these bacteria significantly decreased in \textit{C. lentillifera} after washing, indicating that the bacterial groups were on the surface of \textit{C. lentillifera} and relatively easy to elute by tap water. In the current study, \textit{Vibrio} was one of the representatives of high-dimensional biomarker genera associated with the S group. It significantly decreased abundance in \textit{C. lentillifera} after washing, which benefits consumers’ health, as it could cause seafood-borne diseases (Figure 3). For example, \textit{Vibrio cholerae} is the pathogen causing human cholera. These ancient and widespread infectious diseases have caused many epidemics worldwide, mainly manifested as severe vomiting, diarrhea, water loss, and high mortality, and are considered an international quarantine infectious disease [51]. \textit{V. parahaemolyticus} is another species belonging to the \textit{Vibrio} genera. Eating food containing these bacteria can cause food poisoning, also known as halophilic bacteria food poisoning, the main clinical symptoms of which are acute onset, abdominal pain, vomiting, diarrhea, and watery stool [52].

Although LEfSe analysis revealed that the abundances of some bacteria groups associated with \textit{C. lentillifera} were significantly decreased after washing with tap water, there was only a marginal reduction in both richness and diversity of the entire bacterial communities according to the results of \( \alpha \)-diversity (Table 2). It was notable that there were 233 OTUs specific for the SW group (Figure S1), which may come from the tap water microbiome used for washing, or it may be caused by individual differences. The reduction in the abundance of harmful bacteria (such as \textit{Vibrio}) in this study showed that washing \textit{C. lentillifera} with tap water had a certain positive significance for food safety. In addition, there were still relatively abundant bacterial communities in \textit{C. lentillifera} after washing with tap water, which may have hidden dangers to food safety, and more effective cleaning methods need to be explored. However, the results obtained by high-throughput sequencing in this
For instance, the abundance of Verrucomicrobia, Bacteroidetes, Planctomycetes, Cyanobacteria, Actinobacteria, Verrucomicrobia, Chloroflexi, and Acidobacteria. The predominant prokaryotic phyla were bacterial communities across the seasons in C. lentillifera. The abundance of Bacteroidetes increase with the temperature of seasons, with no significant temporal shifts. Slight changes in the dominant bacterial groups contributed most of the dissimilarity in bacterial communities across the seasons in C. lentillifera. For instance, the abundance of Planctomycetes in C. lentillifera was significantly increased in summer than in the other three seasons, which occurred mostly due to the increase of Planctomycetaceae. The increased abundance of Labrenzia in washed C. lentillifera suggested that there was a symbiotic relationship between C. lentillifera and Labrenzia. In contrast, the significant reduction in the abundance of harmful bacteria (such as Vibrio) showed that washing C. lentillifera with tap water is beneficial for human health. However, we found that both the richness and diversity of the bacterial communities associated with C. lentillifera only slightly decreased after washing with tap water, indicating hidden dangers in C. lentillifera for food safety, and more effective cleaning methods need to be explored.

5 Conclusion

In this study, a total of 4,388 OTUs were obtained from all the samples, and 5 major genera and 14 major classes of bacteria were detected in 43 phyla in C. lentillifera. The predominant prokaryotic phyla were Proteobacteria, Planctomycetes, Cyanobacteria, Actinobacteria, and Acidobacteria. We demonstrated that the bacterial diversities associated with C. lentillifera increase with the temperature of seasons, with no significant temporal shifts. Slight changes in the dominant bacterial groups contributed most of the dissimilarity in bacterial communities across the seasons in C. lentillifera. For instance, the abundance of Planctomycetes in C. lentillifera was significantly increased in summer than in the other three seasons, which occurred mostly due to the increase of Planctomycetaceae. The increased abundance of Labrenzia in washed C. lentillifera suggested that there was a symbiotic relationship between C. lentillifera and Labrenzia. In contrast, the significant reduction in the abundance of harmful bacteria (such as Vibrio) showed that washing C. lentillifera with tap water is beneficial for human health. However, we found that both the richness and diversity of the bacterial communities associated with C. lentillifera only slightly decreased after washing with tap water, indicating hidden dangers in C. lentillifera for food safety, and more effective cleaning methods need to be explored.

Acknowledgments: We thank the technical support from the Beijing Genomics Institute.

Funding information: This research was supported by Shenzhen Science and Technology Project “Studies on cultivation ecology of macroalgae with high value and key basic questions about food safety” (JCY(20170818140317993)), Post-doctoral Foundation Project of Shenzhen Polytechnic (6019330003K), and Post-doctoral Foundation Project of Shenzhen Polytechnic (6019330004K).

Author contributions: Conceptualization: Zhili Huang, and Gang Jin; validation: Meixia Pang, Le Lv, and Xiaodong Li; resources: Zhili Huang; data curation, Meixia Pang; writing – original draft preparation: Meixia Pang; writing – review and editing: Gang Jin; and funding acquisition: Gang Jin and Meixia Pang. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Aguilar-Santos G, Doty M. Caulerpa as food in the Philippines. Philippine Agriculturist. 1968;52:477–82.
[2] Perryman SE, Lapong I, Mustafa A, Sabang R, Rimmer MA. Potential of metal contamination to affect the food safety of seaweed (Caulerpa spp.) cultured in coastal ponds in Sulawesi. Indonesia Aquacult Rep. 2017;5:27–33. doi: 10.1016/j.aqrep.2016.12.002.
[3] Elbashir S, Parveen S, Schwarz J, Rippen T, Jahncke M, DePaola A. Seafood pathogens and information on antimicrobial resistance: a review. Food Microbiol. 2018;70:85–93. doi: 10.1016/j.fm.2017.09.011.
[4] Trono GC. Diversity of the seaweed flora of the Philippines and its utilization. Hydrobiologia. 1999;398:1–6. doi: 10.1023/A:1017097226330.
[5] Hong DD, Hien HM, Son PN. Seaweeds from Vietnam used for functional food, medicine and biofertilizer. J Appl Phycol. 2007;19(6):817–26. doi: 10.1007/s10811-007-9228-x.
[6] Nagappan T, Vairappan CS. Nutritional and bioactive properties of three edible species of green algae, genus Caulerpa (Caulerpaceae). J Appl Phycol. 2016;28(2):1019–27. doi: 10.1007/s10811-013-0147-8.
[7] Matanjun P, Mohamed S, Mustapa NM, Muhammad K. Nutrient content of tropical edible seaweeds, Eucheuma cottonii, Caulerpa lentillifera and Sargassum polycystum. J Appl Phycol. 2009;21(1):75–80. doi: 10.1007/s10811-008-9326-4.
[8] Nguyen VT, Ueng JP, Tsai GJ. Proximate composition, total phenolic content, and antioxidant activity of sea grape Caulerpa lentillifera). J Food Sci. 2011;76(7):C950–8. doi: 10.1111/j.1750-3841.2011.02289.x.
[9] Paul NA, Neveux N, Magnusson M, De Nys R. Comparative production and nutritional value of “sea grapes” – the tropical green seaweeds Caulerpa lentillifera and C. racemosa. J Appl Phycol. 2014;26(4):1833–44. doi: 10.1007/s10811-013-0227-9.
[10] Saito H, Xue C, Yamashiro R, Moromizato S, Itabashi Y. High polyunsaturated fatty acid levels in two subtropical macroalgae, Cladosiphon okamuranus and Caulerpa lentillifera. J Phycol. 2010;46(4):665–73. doi: 10.1111/j.1529-8817.2010.00848.x.
[11] Zhang M, Ma Y, Che X, Huang Z, Chen P, Xia G, et al. Comparative analysis of nutrient composition of Caulerpa lentillifera from different regions. J Ocean U China. 2020;19(2):439–45. doi: 10.1007/s11802-020-4222-x.
[12] Kumar P, Kumar M, Gupta V, Reddy C, Jha B. Tropical marine macroalgae as potential sources of nutritionally important PUFAs. Food Chem. 2010;120(3):749–57. doi: 10.1016/j.foodchem.2009.11.006.

[13] Kumar M, Gupta V, Kumari P, Reddy C, Jha B. Assessment of nutrient composition and antioxidant potential of Caulerpaceae seaweeds. J Food Compost Anal. 2011;24(2):270–8. doi: 10.1016/j.jfca.2010.07.007.

[14] Sharma BR, Rhyu DY. Anti-diabetic effects of Caulerpa lentillifera: stimulation of insulin secretion in pancreatic β-cells and enhancement of glucose uptake in adipocytes. Asian Pac J Trop Bio. 2014;4(7):575–80. doi: 10.12980/APJTB.4.2014APJTB.2014-0091.

[15] Sharma BR, Kim HJ, Rhyu DY. Caulerpa lentillifera extract ameliorates insulin resistance and regulates glucose metabolism in C57BL/KsJ-db/db mice via PI3K/AKT signaling pathway in myocytes. J Transl Med. 2015;13(1):62–71. doi: 10.1186/s12967-015-0415-2.

[16] Sun Y, Liu Z, Song S, Zhu B, Zhao L, Jiang J, et al. Anti-inflammatory activity and structural identification of a sulfated polysaccharide CLGP4 from Caulerpa lentillifera. Int J Biol Macromol. 2020;146:931–8. doi: 10.1016/j.ijbiomac.2019.09.216.

[17] Zhang M, Zhao M, Qing Y, Luo Y, Xia G, Li Y. Study on immunostimulatory activity and extraction process optimization of polysaccharides from Caulerpa lentillifera. Int J Biol Macromol. 2020;143:677–84. doi: 10.1016/j.ijbiomac.2019.10.042.

[18] Joel CH, Sutopo CC, Prajitno A, Su JH, Hsu JL. Screening of angiotensin-I converting enzyme inhibitory peptides derived from Caulerpa lentillifera. Molecules. 2018;23(11):3005. doi: 10.3390/molecules23113005.

[19] Maeda R, Ida T, Ihara H, Sakamoto T. Induction of apoptosis in MCF-7 cells by β-1, 3-xyloligosaccharides prepared from Caulerpa lentillifera. Biosci Biotechnol Biochem. 2012;76(5):1032–4. doi: 10.1271/bbb.120016.

[20] Bonnin-Jusserand M, Copin S, Le Bris C, Brauge T, Gay M, Brisabois A, et al. Vibri species involved in seafood-borne outbreaks (Vibrio cholerae, V. parahaemolyticus and V. vulnificus): review of microbiological versus recent molecular detection methods in seafood products. Crit Rev Food Sci Nutr. 2019;59(4):597–610. doi: 10.1080/10408398.2017.1384715.

[21] Cisneros-Montemayor AM, Pauly D, Weatherdon LV, Ota Y. A global estimate of seafood consumption by coastal indigenous peoples. PLoS One. 2016;11(12):e0166681. doi: 10.1371/journal.pone.0166681.

[22] Karagiannis I, Detsis M, Gkolfinopoulou K, Pervanidou D, Panagiotopoulos T, Bonos A. An outbreak of gastroenteritis linked to seafood consumption in a remote Northern Aegean island, February–March Rural 2010. Remote Health. 2010;10:1507–625. doi: 10.1111/j.1539-6924.2010.01508.x.

[23] Zozaya-Valdes E, Egan S, Thomas T. A comprehensive analysis of the microbial communities of healthy and diseased marine macroalgae and the detection of known and potential bacterial pathogens. Front Microbiol. 2015;6:146. doi: 10.3389/fmicb.2015.00146.

[24] Kudaka J, Ikazaki K, Taiera K, Nidaira M, Okano S, Nakamura M, et al. Investigation and culture of microbial contaminants of Caulerpa lentillifera (sea grape). Shokuhin Eiseigaku Zasshi. 2008;49(1):11–5. doi: 10.3358/shokueishi.49.11.

[25] Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIME allows analysis of high-throughput community sequencing data. Nature methods. 2010;7(5):335–6. doi: 10.1038/nmeth.f.309.

[26] Douglas WF, Bing M, Pawel G, Naomi S, Sandra O, Rebecca MB. An improved dual-indexing approach for multiplexed 16S RNA gene sequencing on the Illumina MiSeq platform. Microbiome. 2014;2(1):6. doi: 10.1186/2049-2618-2-6.

[27] Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27(21):2957–63. doi: 10.1093/bioinformatics/btr507.

[28] Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplion reads. Nat methods. 2013;10(10):996–8. doi: 10.1038/nmeth.2604.

[29] Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194–200. doi: 10.1093/bioinformatics/btr381.

[30] McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 2012;6(3):610–8. doi: 10.1038/ismej.2011.139.

[31] Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microb. 2007;73(16):5261–7. doi: 10.1128/AEM.00062-07.

[32] Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009;75(23):7537–41. doi: 10.1128/AEM.01541-09.

[33] Amato KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A, et al. Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes. ISME J. 2013;7(7):1344–53. doi: 10.1038/ismej.2013.16.

[34] Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32(5):1792–7. doi: 10.1093/nar/gkh340.

[35] Lachnit T, Meske D, Wahl M, Harder T, Schmitz R. Epibacterial community patterns on marine macroalgae are host-specific but temporally variable. Environ Microbiol. 2011;13(3):655–65. doi: 10.1111/j.1462-2980.2010.02371.x.

[36] Lachnit T, Blümel M, Imhoff JF, Wahl M. Specific epibacterial communities on macroalgae: phylogeny matters more than habitat. Aquat Biol. 2009;5(2):181–6. doi: 10.3354/ab00149.

[37] Richards TA, Jones MD, Leonard G, Bass D. Marine fungi: their ecology and molecular diversity. Ann Rev Mar Sci. 2012;4:495–522. doi: 10.1146/annurev-marine-120710-100802.

[38] Tujula NA, Crocetti GR, Burke C, Thomas T, Holmström C, Kjelleberg S. Variability and abundance of the epiphytic bacterial community associated with a green marine Ulva marina. ISME J. 2010;4(2):301–11. doi: 10.1038/ismej.2009.107.

[39] Goecke F, Thiel V, Wiese J, Labes A, Imhoff JF. Algae as an important environment for bacteria—phylogenetic relationships among new bacterial species isolated from algae. Phycology. 2013;52(1):14–24. doi: 10.2216/12-24.1.
[40] Bengtsson MM, Sjøtun K, Øvreås L. Seasonal dynamics of bacterial biofilms on the kelp Laminaria hyperborea. Aquat Microb Ecol. 2010;60(1):71–83. doi: 10.3354/ame01409.

[41] Burke C, Thomas T, Lewis M, Steinberg P, Kjelleberg S. Composition, uniqueness and variability of the epiphytic bacterial community of the green alga Ulva australis. ISME J. 2010;5(4):590–600. doi: 10.1038/ismej.2010.164.

[42] Aires T, Serrão EA, Kendrick G, Duarte CM, Arnaud-Haond S. Invasion is a community affair: clandestine followers in the bacterial community associated to green algae, Caulerpa racemosa, track the invasion source. PLoS One. 2013;8(7):e68429. doi: 10.1371/journal.pone.0068429.

[43] Mancuso FP, D’hondt S, Willems A, Airoldi L, De Clerck O. Diversity and temporal dynamics of the epiphytic bacterial communities associated with the canopy-forming seaweed Cystoseira compressa (Esper) Gerloff and Nizamuddin. Front Microbiol. 2016;7:476. doi: 10.3389/fmicb.2016.00476.

[44] Serebryakova A, Aires T, Viard F, Serrão EA, Engelen AH. Summer shifts of bacterial communities associated with the invasive brown seaweed Sargassum muticum are location and tissue dependent. PLoS One. 2018;13(12):e0206734. doi: 10.1371/journal.pone.0206734.

[45] de Oliveira LS, Gregoracci GB, Silva GG, Salgado LT, Filho GA, Alves-Ferreira M, et al. Transcriptomic analysis of the red seaweed Laurencia dendroidea (Florideophyceae, Rhodophyta) and its microbiome. BMC Genomics. 2012;13(1):487. doi: 10.1186/1471-2164-13-487.

[46] Hollants J, Lelieaut F, De Clerck O, Willems A. What we can learn from sushi: a review on seaweed–bacterial associations. FEMS Microbiol Ecol. 2013;83(1):1–16. doi: 10.1111/j.1574-6941.2012.01446.x.

[47] Lage OM, Bondoso I. Planctomycetes and macroalgae, a striking association. Front Microbiol. 2014;5:267. doi: 10.3389/fmicb.2014.00267.

[48] Bondoso J, Balague V, Gasol JM, Lage OM. Community composition of the Planctomycetes associated with different macroalgae. FEMS Microbiol Ecol. 2014;88(3):445–56. doi: 10.1111/1574-6941.12258.

[49] Biebl H, Pukall R, Lünsdorf H, Schulz S, Allgaier M, Tindall BJ, et al. Description of Labrenzia alexandrii gen. nov., sp. nov., a novel alphaproteobacterium containing bacteriochlorophyll a, and a proposal for reclassification of Stappia aggregata as Labrenzia aggregata comb. nov., of Stappia marina as Labrenzia marina comb. nov. and of Stappia alba as Labrenzia alba comb. nov., and emended descriptions of the genera Pannonibacter, Stappia and Roseibium, and of the species Roseibium denhamense and Roseibium hamelinense. Int J Syst Evol Microbiol. 2007;57(5):1095–1107. doi: 10.1099/ijs.0.64821-0.

[50] Liang Z, Liu F, Wang W, Zhang P, Sun X, Wang F, et al. High-throughput sequencing revealed differences of microbial community structure and diversity between healthy and diseased Caulerpa lentillifera. BMC Microbiol. 2019;19(1):1–15. doi: 10.1186/s12866-019-1605-5.

[51] Weil AA, Becker RL, Harris JB. Vibrio cholerae at the Intersection of Immunity and the Microbiome. Msphere. 2019;4(6):e00597-19. doi: 10.1128/mSphere.00597-19.

[52] Shimohata T, Takahashi A. Diarrhea induced by infection of Vibrio parahaemolyticus. J Med Investigation. 2010;57(3,4):179–82. doi: 10.2152/jmi.57.179.

Bacterial diversities in Caulerpa lentillifera