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How do epiphytic and surrounding seawater bacterial communities shift with the development of the *Saccharina japonica* farmed in the Northern China?

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Epibacteria of seaweeds play an important role for the development of hosts and are influenced by the planktonic surrounding seawater bacteria. However, to date, the knowledges related to both epiphytic and surrounding seawater bacterial communities associated with northern farmed *Saccharina japonica* are very limited. In this study, using 16S rRNA gene amplicon sequencing, the shifts of epiphytic and surrounding seawater bacterial communities of the northern farmed *S. japonica* from mature sporophytes, sporelings (3 time points) to juvenile sporophytes (2 time points) were investigated. The dominant genera of epibacterial communities were *Alcanivorax* (mature sporophytes and 4-week-old sporelings), *Bacillus* (7-week-old sporelings and 9-week-old sporelings), *Halomonas* (4-week-old juvenile sporophytes) and *Cobetia* (9-week-old juvenile sporophytes). Meanwhile, the Chao1 indexes and beta diversity of epibacterial communities were significantly different with the development of *S. japonica* ($p < 0.05$). Furthermore, *Alcanivorax*, *Bacillus* and *Halomonas* were both dominant and core genera, indicating that these taxa may be beneficial to the development of *S. japonica*. The alpha diversity indexes of both epiphytic and surrounding seawater bacterial communities were significantly different for 9-week-old juvenile sporophytes. Therefore, the epibacterial communities were influenced by both development of *S. japonica* and the surrounding seawater bacterial communities. This study not only extends the understanding of the bacterial communities associated with the northern farmed *S. japonica*, but also help to make production management by monitoring the variations in both epiphytic and surrounding seawater bacterial communities.

KEYWORDS

epibacterial communities, surrounding seawater bacterial communities, *Saccharina japonica*, temporal shift, 16S rRNA gene amplicon sequencing, seaweeds

Introduction

Epibacterial communities associated with seaweeds are essential for the development, metabolic functioning and defense of their hosts (Morrissey et al., 2019; Paix et al., 2021) and are affected by physiological and biochemical characteristics of seaweeds during the development, which may lead to selective enrichment of bacterial colonizers on the surfaces (Florez et al., 2019; Saha and Weinberger, 2019). It is found that the dominant genera of epibacterial communities were various at different developmental stages (Mancuso et al., 2016; Comba González et al., 2021) and alpha diversity indexes increased with the development of seaweeds (Michelou et al., 2013; Weigel and Pfister, 2019; Ihua et al., 2020). These studies indicated that the composition and diversity of epibacterial communities shift with the development of seaweeds. In addition, it has been found that core species play the important role in the structure and function of microbial community (Bonthond et al., 2020; Saha et al., 2020; Phelps et al., 2021). Core species analysis has been applied in studies of epibacterial communities of seaweeds to identify bacteria that may contribute to the normal development of host (Tujula et al., 2010; Han et al., 2021; Phelps et al., 2021). Meanwhile, the planktonic bacteria in the surrounding seawater also play an important role in the succession of epibacterial communities associated with seaweeds (Fahimipour et al., 2017; Lemay et al., 2018a; Cleary and Huang, 2020; Juhmani et al., 2020). So far, there is no consistent different or similar conclusions on the relationship between epiphytic and surrounding seawater bacterial communities. All of these outcomes achieved in the wild seaweeds provide the reference to investigate the shifts of epiphytic and surrounding seawater bacterial communities during the development in the commercially farmed seaweeds.

Saccharina japonica is an importantly farmed seaweed worldwide. China contributed about 90% of yield volume globally (Yan et al., 2022). Compared to wild seaweeds, studies on epiphytic and surrounding seawater bacterial communities of farmed *S. japonica* are still at infant stage. Usually, the cultivation period for the northern farmed *S. japonica* in China includes stages of sporelings (from early time of August to mid-October), juvenile sporophytes (from mid-October to next January), adult sporophytes (from February to June) and mature sporophytes with sporangia (from June to early time of August). Using 16S rRNA amplicon sequencing, the epibacterial communities of the northern farmed *S. japonica* were documented at the stages of sporelings, juvenile sporophytes, adult sporophytes and mature sporophytes. The dominant genera, Shannon indexes and beta diversity of the epibacterial communities exhibited significant succession from mature sporophytes, sporelings and juvenile sporophytes (Han et al., 2021) as well as the adult sporophytes during the harvest season (Zhang et al., 2020b). In addition, it is found that the relative abundance of dominant genera *SAR11_clade* and *Candidatus Actinomarina* in the seawater bacterial communities decreased with development of sporelings (Wang et al., 2022). Regarding to the relationship of epiphytic and surrounding seawater bacterial communities during the

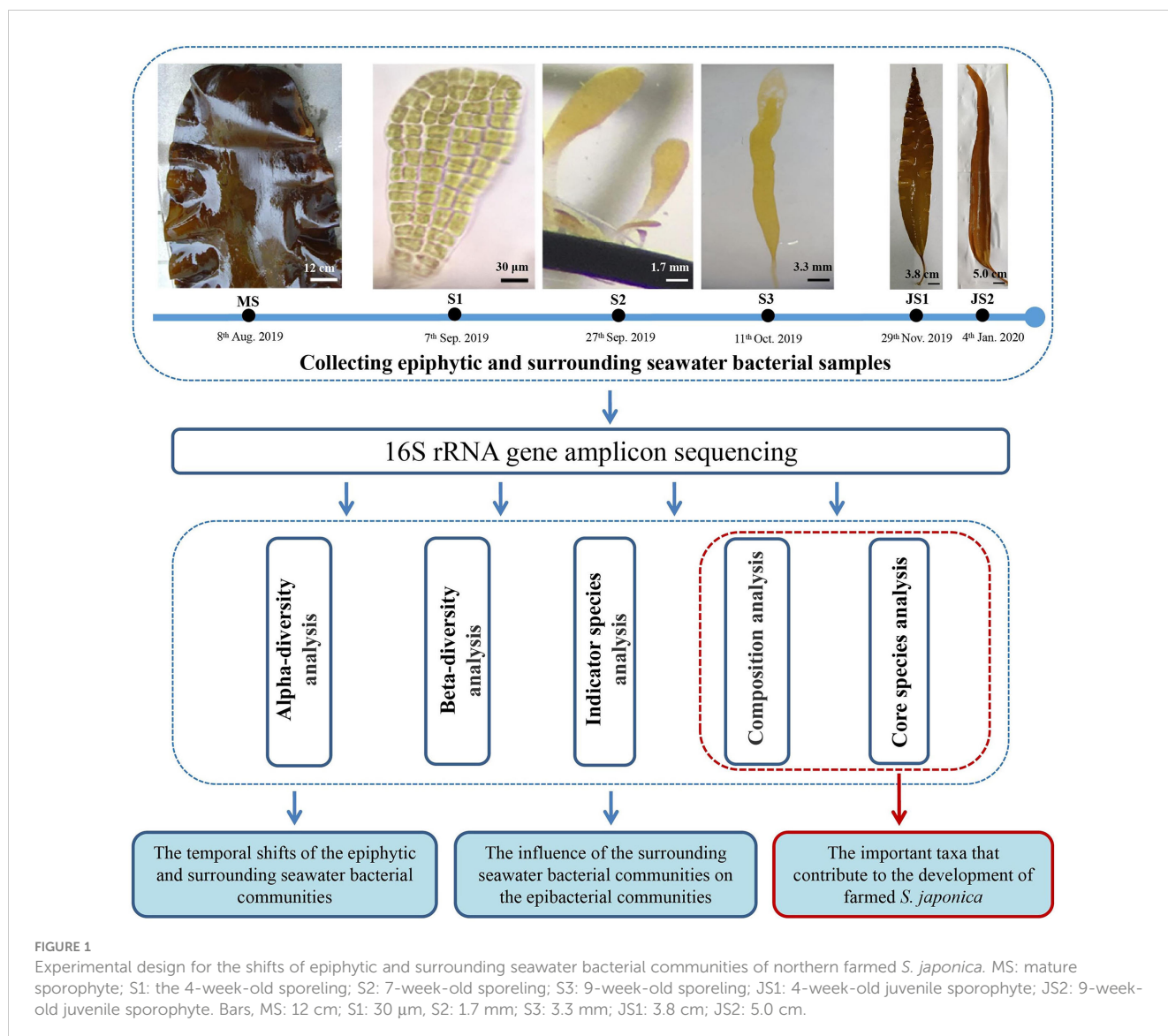
cultivation developmental period, related research was conducted only at the stage of adult sporophytes during the harvest season. The dominant genera were variable and the alpha diversity indexes were different between epiphytic and surrounding seawater bacterial communities (Zhang, 2017), indicating that the epibacterial communities had their own unique bacterial taxa. So far, there are no comprehensive studies on the succession of both epiphytic and surrounding seawater bacterial communities during the cultivation cycle.

Considering that epibacterial communities play crucial roles during the development of seaweeds and are affected by the surrounding seawater bacterial communities, however, the comprehensive knowledges of epiphytic and surrounding seawater bacterial communities in northern farmed *S. japonica* are still unknown. Therefore, we proposed the hypothesis: the shifts of epibacterial communities are influenced by both the development stage of farmed *S. japonica* and the surrounding seawater bacterial communities. Using 16S rRNA gene amplicon sequencing, we first investigated the composition and diversity of the epiphytic and surrounding seawater bacterial communities from mature sporophytes, sporelings to juvenile sporophytes; then we identified the important taxa that contribute to the development of farmed *S. japonica* by dominant and core genera analysis; finally, we assessed the influences of surrounding seawater bacterial communities on the epibacterial communities. Our results not only enrich the knowledge of microbiota associated with northern farmed *S. japonica*, but also will help to develop cultivation management from the angle of monitoring the variations in both epiphytic and surrounding seawater bacterial communities.

Materials and methods

Bacterial sampling

In this study, epiphytic and surrounding seawater bacterial samples were collected at six developmental time points from mature sporophytes, sporelings to juvenile sporophytes stages at Qingyutan, Rongcheng, Shandong Province, China (37°10' 2.19" N, 122°34' 54.8" E). Mature sporophytes (MS) with sporangia were collected on 8th August, 2019 and were rinsed with sterile seawater to remove the loose epiphytes on the surface (Figure 1). Epibacteria were sampled by swabbing 50 cm² of the sporangia surface with sterile cotton swabs near the one-third of mature sporophytes away from the meristematic tissue. The swabs with epibacteria were stored in 50 mL sterile Eppendorf tubes. Usually it takes one month for the zoospores to develop into sporelings (S), which cover the substrate curtains (40 × 115 cm) in the greenhouses. The environmental factors, such as temperature (10 ± 1 °C), salinity (30‰) and light intensity (80 μE m⁻² s⁻¹), were controlled in the greenhouses. Method for sampling epibacterial samples of sporelings referred to Han et al. (2021). Epibacteria of 4-week-old sporelings (S1, 0.1-0.2 mm in length), 7-week-old sporelings (S2, 1-5 mm in length) and 9-week-old sporelings (S3, 10-20 mm in length) were sampled by swabbing on the substrate curtains by



using sterile cotton swabs on 7th September, 27th September and 11th October 2019, respectively (Figure 1). Swabs with epibacteria were put into 50 mL sterile Eppendorf tubes. Juvenile sporophytes (JS) are referred to those sporelings which were transferred into the sea and cultivated for a period of 1-2 month. Epibacterial samples for both 4-week-old juvenile sporophytes (JS1, 30-40 cm in length) and 9-week-old juvenile sporophytes (JS2, 50-60 cm in length) were collected on 29th November 2019 and 4th January 2020, respectively (Figure 1). The sampling method of epibacteria on juvenile sporophytes were the same as mature sporophytes.

Regarding to the collection of seawater bacterial samples, 2.5 L surrounding seawater were collected by using sterile bottles at each developmental time points. Seawater samples were firstly filtered through the membrane of 3 μ m pore size to wash off the larger biotic or abiotic particles, and then were filtered through a membrane of 0.22 μ m pore size (Whatman, UK). These membranes with seawater bacteria were put into 50 mL sterile Eppendorf tubes. All bacterial samples were kept frozen at -80 °C until DNA was extracted. Six

replicates for both epibacterial and seawater bacterial samples were designed for each developmental time points, respectively.

DNA extraction, amplification, and 16S rRNA gene amplicon sequencing

We used HiPure Soil DNA kits (Magen, Guangzhou, China) to extract DNA of bacteria following the manufacturer's instructions. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified by PCR (95°C for 2 min, followed by 27 cycles at 98°C for 10 s, 62°C for 30 s, 68°C for 30 s and a final extension at 68°C for 10 min) with the primers 341F (5'- CCTACGGGNGGCWGCAG-3') and 806R (5'- GGACTACHVGGGTATCTAAT -3'). PCR reactions were performed in 50 μ L mixture, containing 10 \times Buffer KOD (5 μ L), dNTPs (5 μ L, 2 mM), primers 341F and 806R (1.5 μ L of each, 10 μ M), KOD DNA Polymerase (Toyobo, Japan) and 100 ng template DNA.

PCR amplicons were purified from 2% agarose gels using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). Quantification was performed using ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, USA). The 2×250 bp paired-end reads were generated with Guangzhou Genedenovo Biotechnology (China) on the Illumina Novaseq6000 platform (Guo et al., 2017).

Analysis of illumina sequencing data

The DADA2 (version 1.14) was used to denoise and remove low-quality reads (Callahan et al., 2016). Then paired end denoised reads were merged as tags with a minimum overlap of 12 bp. Chimera sequences were identified and were deleted by UCHIME algorithm (Edgar et al., 2011). The high-quality tags were clustered into ASVs (amplicon sequence variants) with 100% similarity. The confidence threshold values ranged from 0.8 to 1.0. All ASV sequences were classified into organisms by a naive Bayesian model using RDP classifier 2.2 (Wang, 2007) based on SILVA database (version 132) (Pruesse et al., 2007). Because there were much higher standard errors in some groups, we removed the three replicates with outliers. Thus, finally, only three replicates close to the average value were selected for the analysis in this study. The sequences of chloroplast, mitochondria and archaea origin were removed from the dataset.

Core species analysis

Genera present in all replicates of epiphytic or surrounding seawater bacterial communities were selected and defined as the core genera. Core genus analysis of both epiphytic and surrounding seawater bacterial communities were performed in R project Venn Diagram package 1.6.16 (Chen and Boutros, 2011). Further, top core genera were identified by screening the core genera with relative abundance more than 1.0%, and was visualized in R project ggplot2 package 2.2.1 (Wickham, 2011).

Statistical analysis

The stacked bar plot of the communities composition was visualized in R project ggplot2 package 2.2.1 (Wickham, 2011). The Chao1, Pielou's and Shannon diversity indices were calculated in QIIME (version 1.9.1) and the comparison between samples were calculated by Welch's test. Non-metric multidimensional scaling (NMDS) and Permutational multivariate analysis of variance (PERMANOVA) were calculated based on unweighted unfrac dissimilarities in R project Vegan package 2.5.3.

Indicator species were those genera which influence structure differences in both epiphytic or surrounding seawater microbial communities. Indicator species were analyzed by calculating the indicator value (*IndVal*) in labdsv package (version 2.0-1) of R project and performing cross-validation test (Roberts, 2016) based on the abundance and occurrence frequency at genus level. Genera

with *IndVal* ≥ 0.7 and *p* value ≤ 0.05 was selected as indicator genus (Glasl et al., 2019).

Results

The compositional variation of epiphytic and surrounding seawater bacterial communities

There were 18 epibacterial and 18 seawater bacterial samples were analyzed from mature sporophytes, sporelings to juvenile sporophytes of northern farmed *S. japonica* (Figure 1). A total of 4,653,122 paired-end raw reads and 4,499,030 clean reads were obtained after filtering low quality reads and removing the mitochondria, chloroplasts and eukaryotes sequences. The 24,821 amplicon sequence variants (ASVs) and were assigned across 36 samples. Among the samples, 14,035 ASVs were clustered in the epibacterial (EB) group, which much higher than those in the seawater (SW) group (13,406 ASVs). Only 10.6% ASVs were shared between epiphytic and seawater bacterial communities (Supplementary Figure 1). ASVs were classified into 41 phyla, 111 classes, 247 orders, 372 families and 958 genera. The rarefaction curves showed good diversity coverage ($> 99.2\%$), indicating the sequencing amount was sufficient (Supplementary Figure 2).

Temporal shifts of epibacterial communities associated with northern farmed *S. japonica* were analyzed from mature sporophytes to juvenile sporophytes (Figure 2). In the epibacterial communities, Proteobacteria (mean relative abundance: 49.1% to 71.1%) was the most dominant phylum at each time point (Figure 2A). At the genus level, *Alcanivorax* was the most dominant genus at both MS-EB (20.4%) and S1-EB (5.5%). *Bacillus* was the most dominant at S2-EB (19.9%) and S3-EB (17.0%). *Halomonas* and *Cobetia* were the most dominant genera at JS1-EB (12.1%) and JS2-EB (15.5%), respectively (Figure 2B). Regarding to the surrounding seawater bacterial communities, the most dominant phylum was Firmicutes at MS-SW (85.8%) and S3-SW (33.5%). Actinobacteria was the most dominant phylum at S1-SW (51.5%). Meanwhile, Proteobacteria was the most dominant phylum at S2-SW (42.2%), JS1-SW (71.8%) and JS2-SW (52.5%). From mature sporophytes, sporelings to juvenile sporophytes, the relative abundance of Firmicutes decreased from 85.8% to 24.6%, and Proteobacteria increased from 6.5% to 62.1% (Figure 2A). At genus level, the most dominant genera were *Virgibacillus* at MS-SW (30.8%), *Rubritalea* at S2-SW (29.2%), *Stenotrophomonas* at JS1-SW (22.0%) and *Cobetia* at JS2-SW (25.2%). *Oceanobacillus* was the most dominant genus at both S1-SW (4.4%) and S3-SW (19.6%) (Figure 2B).

The temporal shifts in diversity of epiphytic and surrounding seawater bacterial communities

There were significant differences in Chao 1 indexes among six developmental time points ($p < 0.05$). Except S1-EB, the Chao 1

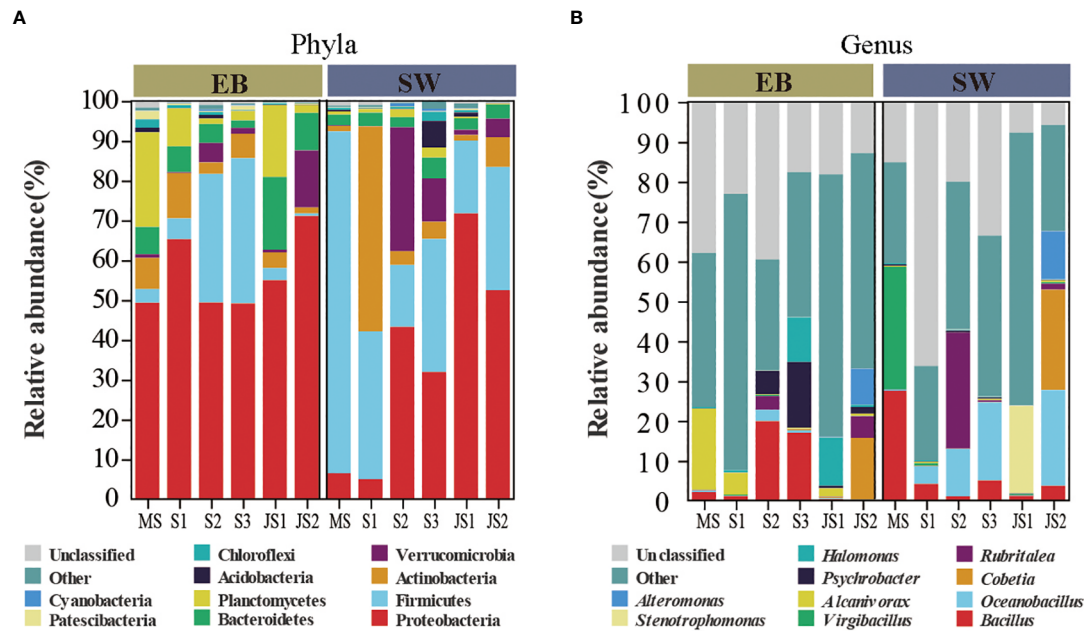


FIGURE 2
The composition of epiphytic and surrounding seawater bacterial communities. MS: mature sporophyte; S1: the 4-week-old sporeling; S2: 7-week-old sporeling; S3: 9-week-old sporeling; JS1: 4-week-old juvenile sporophyte; JS2: 9-week-old juvenile sporophyte. Top 10 ASVs with relative abundance in both epiphytic and seawater bacterial communities at (A) phylum and (B) genus level.

(species richness estimates) indexes of epibacterial communities exhibited increasing trend with the development with northern farmed *S. japonica* (Figure 3A). Regarding to Pielou's evenness and Shannon indexes, there were no significant shifts with the development of *S. japonica* (Figures 3B, C; $p > 0.05$). In

surrounding seawater bacterial communities, Chao 1 indexes were significantly different with development of *S. japonica* (Figure 3D, $p < 0.05$). The Pielou's evenness was not significantly different (Figure 3E, $p > 0.05$), but the Shannon indexes exhibited significant differences (Figure 3F, $p < 0.05$). At the same

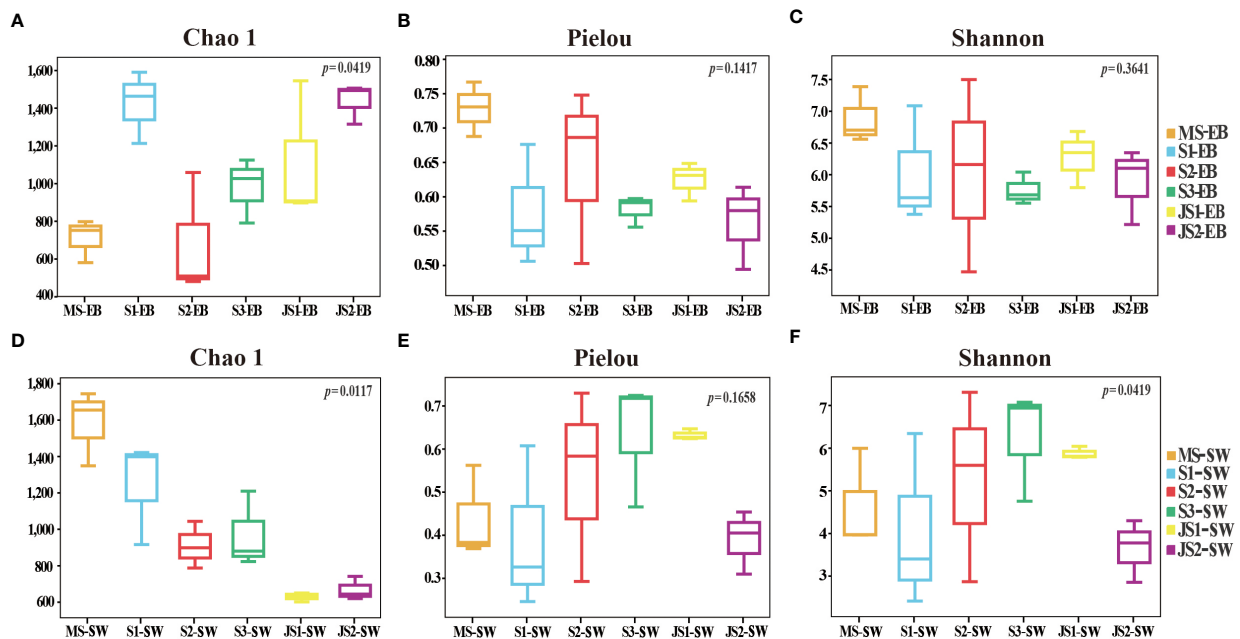


FIGURE 3
Alpha diversity of the epiphytic and surrounding seawater bacterial communities. (A, D): Chao1 species richness; (B, E): Pielou's evenness; (C, F): Shannon diversity indexes.

developmental time point, the Chao 1 indexes (Table 1, $p < 0.01$) and Pielou's evenness (Table 1, $p < 0.05$) of epiphytic and seawater bacterial communities were significantly different at MS and JS2. While, the Shannon indexes of epiphytic and surrounding seawater bacterial communities exhibited significant differences at JS2 (Table 1, $p < 0.05$).

The beta diversity of epibacterial communities displayed significant differences at six developmental time points (Figure 4A). PERMANOVA showed that developmental time

explained 40.8% of epibacterial communities variation (PERMANOVA: F value = 1.66, $R^2 = 0.41$, $P = 0.001$, Table 2). Meanwhile, there were significant differences in the beta diversity of seawater bacterial communities at the different time points (PERMANOVA: F value = 1.89, $R^2 = 0.44$, $P = 0.001$, Table 2; Figure 4B). Moreover, there was no significant difference between the structure of the epiphytic and the surrounding seawater bacterial communities. (PERMANOVA: F value = 1.2, $R^2 = 0.03$, $P = 0.058$, Table 2; Supplementary Figure 3). The structure of

TABLE 1 Alpha diversity of both epiphytic and surrounding seawater bacterial communities at six developmental time points.

Stages	Chao 1		P	Pielou		P	Shannon		P
	EB	SW		EB	SW		EB	SW	
MS	707.94 ± 114.33	1580.71 ± 207.63	0.007	0.73 ± 0.04	0.44 ± 0.11	0.030	6.88 ± 0.44	4.63 ± 1.17	0.066
S1	1419.75 ± 192.02	1243.05 ± 285.61	0.431	0.58 ± 0.09	0.39 ± 0.19	0.229	6.03 ± 0.92	4.04 ± 2.04	0.230
S2	680.73 ± 325.60	907.29 ± 128.93	0.355	0.65 ± 0.13	0.53 ± 0.22	0.506	6.04 ± 1.52	5.25 ± 2.24	0.644
S3	978.31 ± 171.51	968.26 ± 208.62	0.952	0.58 ± 0.02	0.63 ± 0.15	0.592	5.75 ± 0.25	6.25 ± 1.30	0.580
JS1	1114.34 ± 370.77	626.58 ± 25.21	0.150	0.62 ± 0.03	0.63 ± 0.01	0.689	6.27 ± 0.45	5.87 ± 0.14	0.257
JS2	1435.82 ± 106.76	665.73 ± 64.94	0.001	0.56 ± 0.06	0.39 ± 0.07	0.036	5.88 ± 0.59	3.63 ± 0.73	0.016

Boldface represents significant differences, $p < 0.05$.

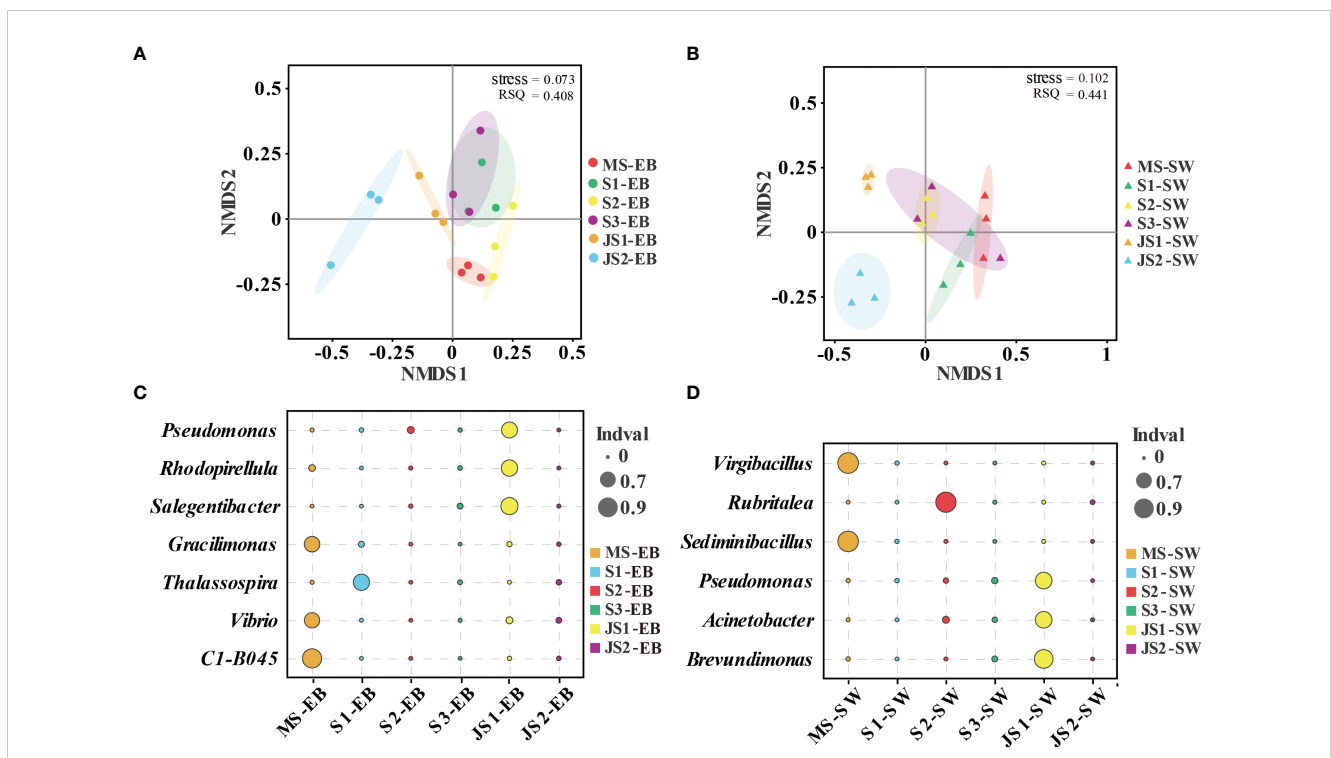


FIGURE 4

The beta diversity and indicator species of the epiphytic and the surrounding seawater bacterial communities at six developmental time points. Non-metric multidimensional scaling (NMDS) plot on amplicon sequence variants (ASV) of (A) epiphytic and (B) surrounding seawater bacterial communities based on the unweighted Unifrac, the circle represented EB group and the triangle represents SW group. The indicator species of (C) epiphytic and (D) surrounding seawater bacterial communities at six developmental time points, the horizontal and the vertical axes indicating the developmental time points and indicator genera, respectively. The bubble size representing the $IndVal$ of the genus, and the bubble color represents the six developmental time points. MS: mature sporophyte; S1: the 4-week-old sporeling; S2: 7-week-old sporeling; S3: 9-week-old sporeling; JS1: 4-week-old juvenile sporophyte; JS2: 9-week-old juvenile sporophyte.

TABLE 2 Permutational multivariate analysis of variance (PERMANOVA) with adonis based on unweighted unifrac dissimilarities of epiphytic and surrounding seawater bacterial communities by the six developmental time points.

Groups	F value	R ²	P value	Significant
Epibacterial communities: seawater bacterial communities	1.228	0.035	0.058	
Epibacterial communities by time	1.656	0.408	0.001	**
Seawater bacterial communities by time	1.894	0.441	0.001	**

F value: Test value; R²: the degree of interpretation of sample difference by different samples; P value: less than 0.01 indicates the high reliability of this test, ** $p < 0.01$.

epiphytic and surrounding seawater bacterial communities were also not significantly different at the same developmental time point by pairwise comparisons (Supplementary Table 1, $p > 0.05$).

In order to further explore the structure differences of epiphytic or surrounding seawater bacterial communities at six developmental time points, indicator species were analyzed based on $IndVal \geq 0.7$ and $p \leq 0.05$ at genus level. The indicator species of MS-EB were *Gracilimonas*, *Vibrio*, *C1-B045*. *Thalassospira* was indicator species at the S1-EB. Whereas, *Pseudomonas Rhodopirellula* and *Salegentibacter* were the indicator species at JS1-EB. There were no indicator species at S2-EB, S3-EB and JS2-EB (Figure 4C). In addition, bubble plot showed different indicator species at different developmental time point to reflect the structure of temporal differences in surrounding seawater bacterial communities (Figure 4D). *Virgibacillus* and *Sediminibacillus* were indicator species at the MS-SW. *Rubritalea* was indicator species at the S2-SW. *Acinetobacter*, *Brevundimonas* and *Pseudomonas* were indicator species at the JS1-SW.

The core genera of epiphytic and surrounding seawater bacterial communities

In this study, core genera were referred to those that were present in all samples at the developmental time points, regardless of their relative abundance. Overall, there were 48 core genera in the epibacterial communities (Supplementary Figure 4A). Among these core genera, the mean relative abundance greater than 1.0% were referred to top core genera. The top core genera included *Bacillus*, *Alcanivorax*, *Halomonas*, *Psychrobacter*, *Blastopirellula*, *Erythrobacter*, *Rubritalea*, *Pseudomonas*, *SM1A02*, *Muricauda* and *Gimesia* (Table 3). From MS to S3, the relative abundance of *Bacillus* in the epibacterial communities gradually increased (ranging from 0.1% to 17.0%) (Supplementary Figure 5). There were 29 core genera (Supplementary Figure 4B) and 7 top core genera (Table 3) in the seawater bacterial communities. Among these top core genera, the relative abundance of *Bacillus* (27.2%),

TABLE 3 Top core genera with their mean relative abundances of epiphytic and surrounding seawater bacterial communities at six developmental time points.

Genus	Family	EB						SW					
		MS	S1	S2	S3	JS1	JS2	MS	S1	S2	S3	JS1	JS2
<i>Bacillus</i> *	Bacillaceae	0.09	1.09	14.58	16.95	0.32	0.09	27.22	4.12	1.19	5.15	1.10	3.59
<i>Alcanivorax</i>	Alcanivoraceae	20.08	5.48	0.19	0.19	2.23	0.60	0.29	0.33	0.03	0.38	0.02	0.44
<i>Halomonas</i>	Halomonadaceae	0.36	0.74	0.04	11.11	12.14	0.56	0.23	0.27	0.21	0.18	0.04	0.06
<i>Psychrobacter</i>	Moraxellaceae	0.05	0.03	5.55	16.72	0.55	1.62	0.34	0.08	0.46	0.47	0.02	0.11
<i>Blastopirellula</i>	Pirellulaceae	1.57	3.58	0.27	0.50	9.30	0.13	0.06	0.11	0.14	0.75	0.00	0.03
<i>Erythrobacter</i>	Sphingomonadaceae	0.87	3.15	2.89	4.09	1.33	0.34	0.42	0.08	0.26	0.19	0.02	0.13
<i>Rubritalea</i> *	Rubritaleaceae	0.19	0.01	6.02	0.02	0.06	2.89	0.00	0.01	23.38	0.29	0.21	1.55
<i>Pseudomonas</i> *	Pseudomonadaceae	0.13	0.27	1.02	0.22	4.66	0.03	0.29	0.43	1.19	1.77	10.44	0.00
<i>SM1A02</i>	Phycisphaeraceae	1.83	0.77	0.01	0.93	2.63	0.12	0.03	0.04	0.08	0.10	0.00	0.01
<i>Muricauda</i>	Flavobacteriaceae	1.30	0.95	0.01	0.03	3.85	0.09	0.05	0.06	0.04	0.10	0.01	0.01
<i>Gimesia</i>	Gimesiaceae	3.21	1.73	0.17	0.20	0.28	0.48	0.04	0.07	0.47	0.07	0.00	0.00
<i>Virgibacillus</i>	Bacillaceae	0.02	0.28	0.19	0.09	0.00	0.00	31.90	0.78	0.00	0.01	0.35	0.38
<i>Sediminibacillus</i>	Bacillaceae	0.07	0.33	0.06	0.44	0.07	0.01	15.81	0.37	0.29	0.01	0.14	0.12
<i>Acinetobacter</i>	Moraxellaceae	0.35	0.04	1.01	0.15	0.13	2.65	0.12	0.03	2.54	1.28	10.41	0.08
<i>Bradyrhizobium</i>	Xanthobacteraceae	0.22	0.01	0.12	0.32	0.12	0.00	0.01	0.01	4.63	2.96	0.01	0.02

The mean relative abundance of *Rubritalea* at MS-SW was 0.004. However, the mean relative abundance values were retained to two decimal places, so the mean relative abundance of *Rubritalea* was 0.00 at MS-SW in the table. The genera marked with "*" were the top core genera between epiphytic and surrounding seawater bacterial communities.

Virgibacillus (31.9%) and *Sediminibacillus* (15.8%) were higher at MS-SW. While, the relative abundance of *Bradyrhizobium* (4.6%) and *Rubritalea* (23.4%) was higher at both S2-SW (Supplementary Figure 5). *Acinetobacter* (10.4%) and *Pseudomonas* (10.4%) was higher at both JS1-SW. *Bacillus*, *Rubritalea* and *Pseudomonas* were top core genera between epiphytic and surrounding seawater bacterial communities. From MS to S2, the relative abundance of *Bacillus* in the surrounding seawater bacterial communities gradually decreased, which was the opposite of the epibacterial communities (Supplementary Figure 5). The top core genera *Rubritalea* between epiphytic and surrounding seawater bacterial communities had the highest relative abundance at S2, and the highest relative abundance of *Pseudomonas* at JS1. The relative abundance of *Rubritalea* and *Pseudomonas* were higher in the surrounding seawater bacterial communities at S2 and JS1 (Table 3).

Discussion

Compared to the wild seaweeds, epimicrobial communities of farmed seaweeds are still in infancy. This study investigated the temporal shifts of epiphytic and surrounding seawater bacterial communities at six developmental time points of commercially northern farmed *S. japonica* by 16S rRNA amplicon sequencing. These results are consistent with our hypothesis. First, the composition of epibacterial communities shifted with the development of *S. japonica*. Moreover, the Chao1 indexes and beta diversity of epibacterial communities were significantly different with the development of *S. japonica* ($p < 0.05$). Meanwhile, we identified the important bacteria that contribute to the normal development of farmed *S. japonica* by analyzing the dominant and core genera of epibacterial communities. Furthermore, in contrast to the surrounding seawater bacterial communities, the composition and structure of the epibacterial communities were influenced by both host development and the surrounding seawater bacterial communities. To our knowledge, this is the first characterization of temporal shifts and the differences between epiphytic and surrounding seawater bacterial communities in the commercially northern farmed *S. japonica*.

Dominant and core genera of the epibacterial communities

It was widely accepted that dominant taxa in microbial communities of seaweeds were species-specific at genus level (Bengtsson and Øvreås, 2010; Mancuso et al., 2016; Lemay et al., 2018b, Lemay et al., 2021a; Guo et al., 2022). In this study, the dominant genera with the highest relative abundance were *Alcanivorax* (MS-EB and S1-EB), *Bacillus* (S2-EB and S3-EB), *Halomonas* (JS1-EB) and *Cobetia* (JS2-EB) (Figure 2B). Previous studies had shown that *Flavobacterium*, *Yoonia*-*Loktanella* and *Planctomyces* were the most dominant genera for brown alga *Sargassum horneri* (Mei et al., 2019), *Sargassum thunbergii* (Guo et al., 2022) and the farmed *Caulerpa lentillifera* (Pang et al., 2022),

respectively. This difference indicated that dominant genera were seaweeds specific (Roth-Schulze et al., 2016; Korlević et al., 2021). What's interesting, in this study, the dominant genera *Alcanivorax*, *Bacillus* and *Halomonas* were also the core genera. *Alcanivorax* is a degrading bacterium for marine hydrocarbon pollutants (Olivera et al., 2009), which may render its seaweed hosts to decontaminate the hydrocarbon pollutants and contribute to the healthy development. *Bacillus*, especially *Bacillus subtilis*, has strong antibacterial activity against the pathogens *Aeromonas hydrophila*, *Vibrio vulnificus* and *Vibrio parahaemolyticus* of the brown alga *Sargassum myriocystum* (Chakraborty et al., 2017). Moreover, *Halomonas* has been found at the wide range of pH and temperatures as well as at almost any range of salinity, which enables *Halomonas* to colonize the variety of habitats (Kim et al., 2013). *Halomonas* is also beneficial to the morphogenesis and development of seaweeds, which excretes a specific regulator like cytokinins that enhanced cell division (Spoerner et al., 2012). For example, *Halomonas* sp. Z3 has been reported to promote the development and increase the number of individuals of the brown alga *Ectocarpus* sp. (Tapia et al., 2016). Based on the relative abundance advantage and beneficial effects, we speculate that *Alcanivorax*, *Bacillus* and *Halomonas* may play beneficial roles during the development of northern farmed *S. japonica*. However, their effects on the seaweed development still remain to be further verified by isolating the pure bacterial strains.

Temporal shifts in diversity of the epibacterial communities

The temporal shifts of diversity with the development of seaweeds was one of the evidences of microbial communities shifts (Weigel and Pfister, 2019). Previous studies indicate that the alpha diversity gradually increased with the development of *Laminaria saccharina* (Staufenberger et al., 2008), *Cystoseira compressa* (Mancuso et al., 2016) and the *Sargassum muticum* (Serebryakova et al., 2018). Consistent with these previous researches, we found the Chao 1 indexes of epibacterial communities increased and was significantly different with the development of northern farmed *S. japonica* ($p < 0.05$). Meanwhile, beta diversity revealed significant differences in the epibacterial communities among different developmental stages (Figure 4A, $p < 0.01$). The epiphytic bacterial communities exhibited a significant increase in species diversity as well as significant differences in community structure, but the Pielou's evenness was stable during the development of northern farmed *S. japonica* (Figure 3B). This suggested that the epibacterial communities continued to recruit new bacteria to assemble the communities with the development of *S. japonica*, rather than based on the increase in the abundance of already colonized bacteria. In addition, it is reported that different metabolites (e.g., enzymes and phenolics) can be secreted by the seaweeds at different development time points (Collén and Davison, 2001; Malik et al., 2020; Han et al., 2021; Lemay et al., 2021a), which can attract the attachment of certain bacteria (Zheng et al., 2005) and thus have the unique microbial composition and structure at different developmental time points.

Epibacterial communities of *S. japonica* influenced by the surrounding seawater bacterial communities

Studies have shown that epibacterial communities of seaweeds were specific and were influenced by the surrounding seawater bacterial communities (Fahimipour et al., 2017; Lemay et al., 2018a; Cleary and Huang, 2020; Juhmani et al., 2020). It was found that the dominant bacteria of *Macrocystis pyrifera* (Michelou et al., 2013), *Thalassia testudinum* and *Syringodium filliforme* (Ugarelli et al., 2018) were shared between the epiphytic and surrounding seawater communities at the phylum level. Consistent with these previous investigations, we observed that Proteobacteria was the dominant phyla in both epiphytic and surrounding seawater bacterial communities. Moreover, the top core genera in the epibacterial communities were all present in the surrounding seawater bacterial communities, which is similar to the results in *Nereocystis luetkeana* and *M. pyrifera* (Weigel and Pfister, 2019). The top core genera *Rubritalea* and *Pseudomonas* between epiphytic and surrounding seawater bacterial communities had the highest relative abundance at the same developmental time point, and their relative abundance were higher in the surrounding seawater bacterial communities. Meanwhile, the structure of the epiphytic and surrounding seawater bacterial communities did not differ significantly. These results suggested that the surrounding seawater bacterial communities influenced the epibacterial communities. It could be explained by the fact that the surface of *S. japonica* was constantly in contact with the diversified free-living bacteria in the surrounding seawater and most of the epibacteria are recruited from the surrounding seawater (Mancuso et al., 2016; Weigel and Pfister, 2019). It is worth noting that microbial communities of seaweeds have been shown to be influenced by a variety of factors, including host species, geography and environment, and so on (Hollants et al., 2013; Morrissey et al., 2019). Therefore, the factors influencing the shifts of epibacterial communities of the farmed *S. japonica* will need to be explored more fully and deeply in the future.

It has been found that the epibacterial communities associated with seaweeds are also influenced by the seaweeds themselves (Weigel and Pfister, 2019; James et al., 2020; Lemay et al., 2021b). In this study, the alpha diversity of the epibacterial communities, including Chao1, Pielou's evenness and the Shannon indexes, were significantly higher than those of surrounding seawater bacterial communities at JS2 ($p < 0.05$). The reason for the higher alpha diversity at JS2 maybe due to the faster growth of *S. japonica*. Zimmerman and Kremer (1986) and Zhang et al. (2020a) reported that the concentrations of mannitol and laminarin on *S. japonica* reduced when it grew fast. Therefore, we speculate that the decreased concentrations of mannitol and laminarin on *S. japonica* at JS2 may attract or inhibit bacterial attachment and then led to a higher alpha diversity of the epibacterial communities. This indicated that the epibacterial communities associated with *S. japonica* were also influenced by the seaweeds themselves. Our results were consistent with previous findings obtained from *Ecklonia radiata* (Marzinelli et al., 2015), *Cystoseira compressa* (Mancuso et al., 2016) and *Caulerpa taxifolia* (Morrissey et al., 2019). This suggested that the epibacterial communities were more

affected by the host itself than the surrounding seawater bacterial communities at the faster growth stage of *S. japonica*.

Conclusions

Using 16S rRNA gene amplicon sequencing, we investigated the shifts of epiphytic and surrounding seawater bacterial communities of northern farmed *S. japonica* from mature to juvenile sporophyte stages. Our results indicated that the dominant genera of epibacterial communities shifted with the development of *S. japonica*. Moreover, the Chao1 indexes and beta diversity of epibacterial communities were significantly different among the six developmental time points. Combining analysis of the dominant and core genera, *Alcanivorax*, *Bacillus* and *Halomonas* may contribute to the development of northern farmed *S. japonica*. Finally, the epibacterial communities were influenced by both *S. japonica* itself and the surrounding seawater bacterial communities. These findings provide novel insights into the bacterial communities associated with northern farmed *S. japonica*.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/>, PRJNA903939.

Author contributions

GW conceived, designed the experiment and revised the manuscript. LCA analyzed the sequencing data and wrote the manuscript. XG involved in the analysis of sequencing data. MS participated in sequencing analysis and helped to improve the English writing. YH collected samples of *Saccharina japonica*. LCh and LX provided the samples of *S. japonica*. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author LCh and LX are employed by Weihai Changqing Ocean Science & Technology Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or

financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1117926/full#supplementary-material>

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