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Comparative Metagenomic Analysis of Marine eDNA Investigating the Production Crisis of Aquacultured *Saccharina japonica*

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Abstract: Aquaculture farms cultivating *Saccharina japonica* are highly active in Wando, Korea, and Rongcheng, China. However, the yield of *S. japonica* significantly declined in the Rongcheng region in 2022 compared to previous records, whereas that in Wando remained at a normal level, presumably due to the presence of a pathogenic microbiome. We used environmental DNA (eDNA) metagenomic analysis to compare the microbial compositions of seawater from aquaculture farms in Wando and Rongcheng. Seawater samples were collected from one Korean site in Wando (WA) and two Chinese sites in Ailian Bay (AB) and Lidao Bay (LB). Metagenomic analysis focusing on the microbial 16S rRNA identified 38 phyla and 58 families of microbiomes in all regions. Potentially pathogenic bacterial groups associated with *S. japonica* in AB and LB were more abundant than in WA, suggesting their potential influence on mortality and the decline in the harvest yield of *S. japonica*. The microbial composition of WA was distinguished from those of the other two sites, which clustered together with higher similarity. Since the *S. japonica* aquaculture industry is important for both countries, this comparative eDNA monitoring is a valuable initiation towards the next step of problem-solving practices in coastal management in these two aquaculture systems.

Keywords: environmental DNA (eDNA); kelp; microorganism; pathogenic bacteria; metagenomic analysis

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1. Introduction

Saccharina japonica is a kelp species that is actively cultivated in Korea and China [1,2]. Specifically, Wando in Korea and Rongcheng in China serve as two prominent regions for the aquaculture of *S. japonica* in these countries [3,4]. Wando produces approximately 97% of the kelp in Korea, and Rongcheng produces approximately 40% of the kelp in China; it is also one of the largest kelp aquaculture farms in China, and both regions contribute to the highest proportion of the global kelp harvest yield [5–7]. The average annual production is estimated to be approximately 1,650,000 tons and 440,000 tons in Rongcheng and Wando, respectively [8,9]. Moreover, these two regions are situated in the Yellow Sea, which lies between the Chinese mainland and the Korean Peninsula and share many temperate marine environments. *Saccharina japonica* is one of the most important cultivated marine

resources and is widely used in a variety of industries, including the food, cosmetics, and pharmaceutical industries [10–12]. Therefore, sustaining harvest conditions and yields of *S. japonica* is important.

However, around February 2022, the necrosis of blades and the large-scale dislodgement of juveniles from the cultivation substrates were observed in *S. japonica* cultivation farms in Rongcheng. As a result, according to the Rongcheng Municipal Government Fishery Statistical Yearbook, the 2022 average production in Rongcheng was 79% lower (about 0.35-million-ton wet weight) compared to the average production (1.65-million-ton wet weight) in 2020 and 2021. In contrast, the harvest yield of *S. japonica* from aquaculture farms in Wando, Korea, remained at the usual level. It has been reported that unexpected decreases in the harvest yield of cultured *S. japonica* are often linked to various environmental factors and the relationship between microorganisms, resulting in disease outbreaks [7,13–15]. In addition to epiphytic organisms, microbial communities in seawater play an important role in the health and growth of kelp and seaweed [16,17]. Various species of pathogenic bacteria and associated diseases of kelp have been reported [7,18].

Microbial communities in seawater can be examined using environmental DNA (eDNA) metagenomic analyses [19]. Using eDNA, it is possible to monitor invisible microorganisms within specific environments as a snapshot observation of a particular point in time [20]. Various studies have used the metagenomic analysis of eDNA to investigate microorganisms in seawater [21,22]. Similarly, eDNA analysis can be applied to study the microbial communities in the seawater of kelp aquaculture farms. Previous studies on the pathogenic bacteria that cause kelp disease have mainly focused on epiphytic bacteria present in kelp tissues [5,23,24]. However, research on epiphytic bacteria from kelp is limited to the isolation and culturing of various pathogenic bacteria. To overcome these limitations and identify the effects of microorganisms present not only on the surface of kelp, but also in the seawater of aquaculture farms, eDNA-based approaches towards the analysis of seawater would improve community characterization. Furthermore, few studies providing snapshot comparisons of the microbial communities of kelp aquaculture farms using eDNA from seawater have been conducted to date.

We began the present study with the assumption that the massive mortality issue observed in Chinese kelp aquaculture farms could be related to the microbial community of seawater because the extent of occurrence was on a regional scale (i.e., south and east coasts of Shandong Province), and there were no detectable pre-symptoms or historical records of the massive mortality of cultivated kelp. If there are any distinct microbial composition patterns in the seawater of Rongcheng, China, in comparison with Wando, Korea, as a reference site, it could provide useful information that would help determine the direction of research for further studies. Therefore, we conducted a comparative analysis of microbial assemblages in kelp aquaculture farms in these two regions using an eDNA metagenomic approach. Furthermore, to identify the potential causes of the decrease in kelp harvest yield, we compared the presence and abundance of pathogenic bacteria in each region. This will help determine the direction of research on the identification of substantive causes of kelp mortality.

2. Materials and Methods

2.1. Sample Collection and Extraction of eDNA

Seawater samples were obtained from aquaculture farms of *S. japonica* in Wando, Korea, and Rongcheng, China (Table S1). Fourteen samples were collected from three regions: Bogilmyun, Wando, Korea (WA; 34.139535° N, 126.613365° E); Ailian Bay, Rongcheng, China (AB; 37.182014° N, 122.610724° E); and Lidao Bay, Rongcheng, China (LB; 37.339227° N, 122.695365° E) (Figure 1). Sampling at all sites was conducted from 1 to 2 December 2022. The water temperature on sampling days was 15.08 °C (range: 9.04–20.49 °C) in WA, 12.80 °C (range: 2.50–24.20 °C) in AB, and 13.30 °C (range: 3.10–23.70 °C) in LB. The salinity was 33.00 PSU (range 32.43 to 33.20 PSU) in WA, 31.80 PSU (range 31.10 to 32.50 PSU) in AB, and 31.75 PSU (range 31.45 to 32.05 PSU) in LB. The abiotic

parameters were determined using an open data pool from the Korea Maritime Institute (https://www.kmi.re.kr, accessed on 26 December 2022) and Weihai Ocean Marine Forecast (https://mp.weixin.qq.com/mp/appmsgalbum?__biz=MzI5MzgwMDMzOQ==&action=getalbum&album_id=2365851694200291329&scene=173&from_msgid=2247543265&from_itemidx=1&count=3&nolastread=1#wechat_redirect, accessed on 26 December 2022). Seawater samples (2 L) were collected from a depth of 1.5 m at 4–5 sites in each region, using a vertical water sampling bottle. The collected seawater samples were stored in an ice-cooler in the field and, upon being delivered to the lab, were immediately stored at −20 °C in the lab until filtration and DNA extraction. All samples were filtered within 48 h after being frozen. Filtration of each 2 L sample was performed using a cellulose acetate filter with a pore size of 0.22 µm (Corning, New York, NY, USA). eDNA extraction from the filter was performed immediately after filtering using a FastDNA™ SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). Samples were eluted in 100 µL of DNAse Free Water (DES). The quality and concentration of extracted DNA were assessed using a Nanophotometer®® NP80 spectrophotometer (Implen, München, Germany).

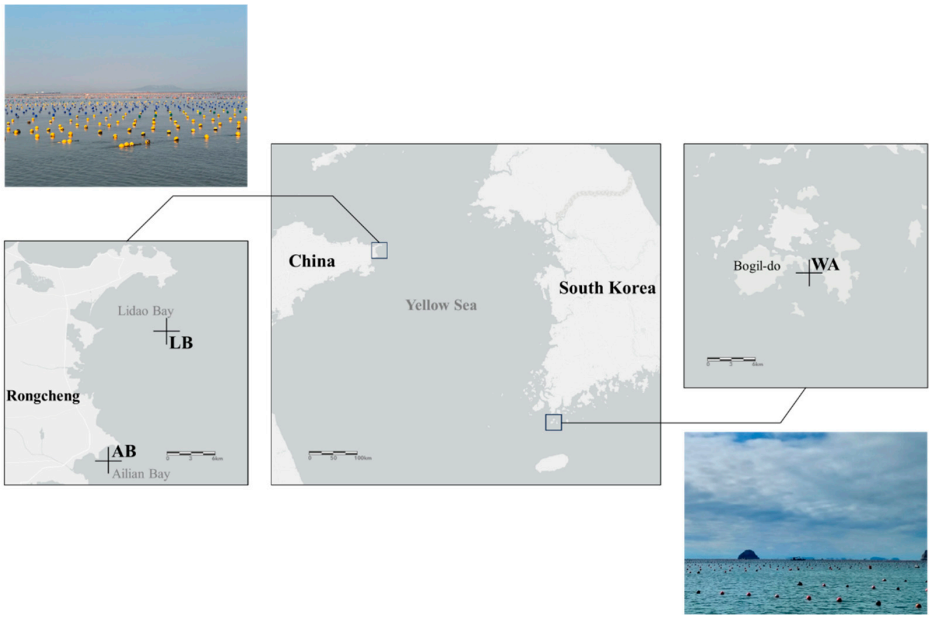

Figure 1. Sampling sites of seawater samples of Korea and China: Bogil-do: Wando, Korea (WA); Ailian Bay, Rongcheng China (AB); Lidao Bay, Rongcheng China (LB).

2.2. Amplification of 16S rRNA and Sequencing

PCR amplification of the extracted DNA was performed to analyze the microbial communities. The V3-V4 region of the bacterial 16S rRNA gene was amplified using the universal primer set (341F 5′-CCTACGGGNGGCWGCAG-3′ and 785R 5′-GACTACHVGGGTATCTAATCC-3′), tagged with the adapter and index sequences for constructing the sequencing library of the Illumina Miseq platform (Illumina, San Diego, CA, USA). The PCR reaction mixture (50 µL) contained 50 ng of template DNA, 1 µL of each primer (20 pmol), 25 µL of 2X EmeraldAmp Max PCR Master Mix (Takara Bio, Tokyo, Japan), and double-distilled water for the rest. PCR conditions were as follows: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 2 min, with a final extension step at 72 °C for 5 min. The size of PCR products was identified by electrophoresis on 1.5% agarose gel (approx. 444 bp) and then purified using a LaboPass™ Gel and PCR Clean-up Kit (Cosmo Genetech, Seoul, South Korea). Purified products were quantified using the qPCR Quantification Protocol Guide (KAPA Library Quantification kits for Illumina Sequencing platforms) and qualified using the LabChip GX HT DNA High Sensitivity Kit (Perkin Elmer, Boston, MA, USA). The paired-end (2 × 300 bp) sequencing was performed on the Illumina Miseq™ platform (Illumina, San Diego, CA, USA).

2.3. Bioinformatic Data Analysis

Metagenomic microbial community analysis of the demultiplexed reads was performed using Quantitative Insights into Microbial Ecology 2 (QIIME2 2023.2; https://qiime2.org/, accessed on 25 August 2023) [25]. Non-biological sequences such as primers were trimmed using the Cutadapt plugin [26]. Sequence reads were clustered into operational taxonomic units (OTUs). OTUs were clustered de novo with 97% sequence similarity using the VSEARCH algorithm [27]. Chimeric reads were removed from the OTUs using the UCHIME de novo plugin, which is a QIIME2 subcommand of the VSEARCH plugin [28]. The taxonomic profiling of the chimera-removed OTUs was performed using the SILVA database. Mitochondrial and chloroplast reads were filtered from the OTUs and taxonomic assignment results.

To analyze seawater microbial communities, the taxonomic microbial composition of each sample was assessed at the phylum and family levels. The alpha diversity of the microbiome in seawater samples was assessed by observing features; Chao1; Faith phylogenetic diversity (Faith PD); Abundance-based Coverage Estimation (ACE); and Simpson, Shannon, and Pielou's evenness indices. Beta diversity was assessed through principal coordinate analysis (PCoA) based on the Jaccard similarity distance and PERMANOVA statistical method. PERMANOVA was performed with the vegan package in R (4.3.2), based on the sample group (three sampling regions). The results of the metagenomic analysis were visualized on a plot using RStudio (2023.12.0+369).

3. Results

3.1. Sequencing Results of 16S rRNA Obtained from eDNA

The sequencing of bacterial 16S rRNA amplicons was performed on seawater samples. To obtain the final dataset, we filtered out OTUs with fewer than five occurrences. The numbers of OTUs and reads produced from the sequencing results of the WA, AB, and LB samples are presented in Table S2. A total of 24,670 OTUs and over 354 million raw reads were obtained from all the samples. The OTUs belonged to 2138 species, 1217 genera, 672 families, 436 orders, 177 classes, and 76 phyla of microorganisms (Table S3). To determine the correlation between the number of sequences and OTUs, rarefaction and extrapolation curves for each sample were identified based on the sequencing results (Figure S1). These curves exhibited a saturation phase beyond a certain threshold, providing confidence in the analysis and indicating that the number of sequence reads was sufficient to represent the clustering distribution of biological species.

3.2. Taxonomic Microbial Communities of Seawater from Wando and Rongcheng

The taxonomic microbial composition and relative abundance at the phylum and family levels for each sample are shown in Figure 2. A total of 38 distinct phyla and 58 distinct families were identified. At the phylum level, Proteobacteria (average 61.57%) was the most abundant taxon in all regions. In WA samples, Marinimicrobia were more abundant than in the other regions, whereas in AB samples, Planctomycetota were more abundant than in the other regions (Figure 2a). At the family level, *Gimesiaceae* and *Psychromonadaceae*, *Nitrincolaceae*, and *Thioglobaceae* were more abundant in the AB, LB, and WA samples, respectively (Figure 2b).

Among the potentially pathogenic bacteria affecting *S. japonica*, four major bacterial genera (*Pseudoalteromonas*, *Vibrio*, *Pseudomonas*, and *Sulfitobacter*) were detected in all regions. In terms of the proportions of these genera relative to the total counts in each sample, these pathogenic bacteria were more abundant in the Chinese seawater samples AB and LB than in WA. Specifically, *Pseudoalteromonas* and *Vibrio* were mainly detected in the LB4 (2016 counts) and AB3 samples (2870 counts), respectively. *Pseudomonas* was commonly detected in WA4 (434 counts) and WA5 (445 counts); however, it was also detected in AB samples (average of 725 counts), especially in AB2 (1513 counts). *Sulfitobacter* was the least frequently detected pathogenic bacteria in all regions but was more abundant in LB samples (average 53 counts) than in the other regions (Figure 2c).

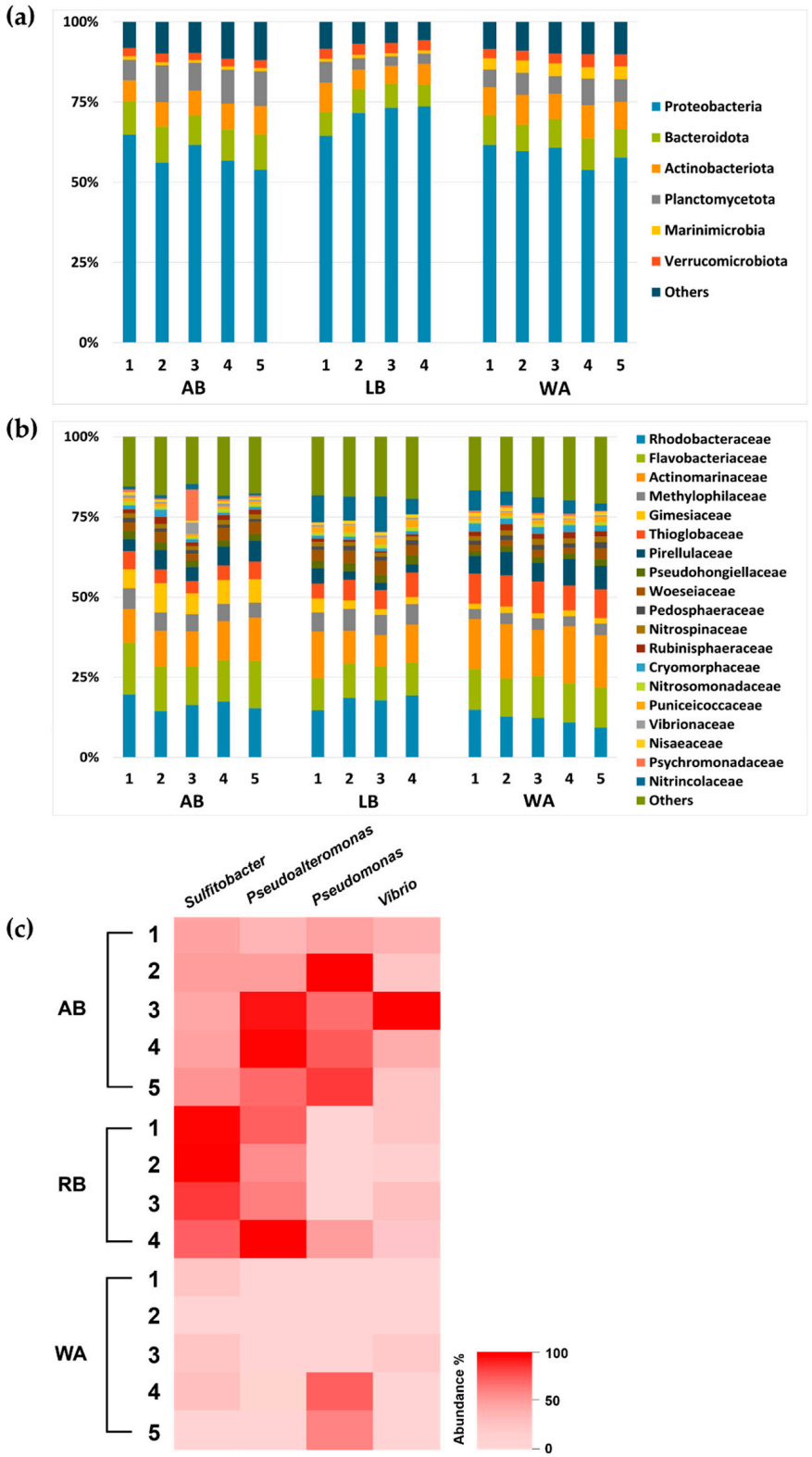

Figure 2. Taxonomic microbial composition and relative abundance at (**a**) phylum and (**b**) family level in each seawater sample. (**c**) Heatmap legend shows the ratio of the tentative pathogenic bacteria in genus level relative to the total counts in AB, LB, and WA. Four major tentatively pathogenic bacteria, *Pseudoalteromonas*, *Vibrio*, *Pseudomonas*, and *Sulfitobacter,* were more abundant in the China seawater samples, AB and LB.

3.3. Comparison of Microbial Diversity between the Seawater Samples

The diversity of the microbial communities in seawater was assessed using alpha and beta diversity analyses (Figure 3). Microbial species diversity was measured using alpha diversity indices, which included Observed Features, Chao1, Faith PD, and ACE for species richness, and Simpson, Shannon, and Pielou's evenness for species richness with evenness. By comparing the alpha diversity values of the three regions, WA showed the highest species diversity among all the regions, and species evenness was generally high in AB and WA. LB had the lowest microbial diversity and evenness (Figure 3a). The beta diversity measured by PCoA based on the Jaccard similarity distance (PERMANOVA: F-value = 2.265; p-value < 0.05) showed that the seawater microbiota of AB, LB, and WA samples were distinctly separated from each other, with a more remarkable dissimilarity between Korean and Chinese seawater samples (Figure 3b).

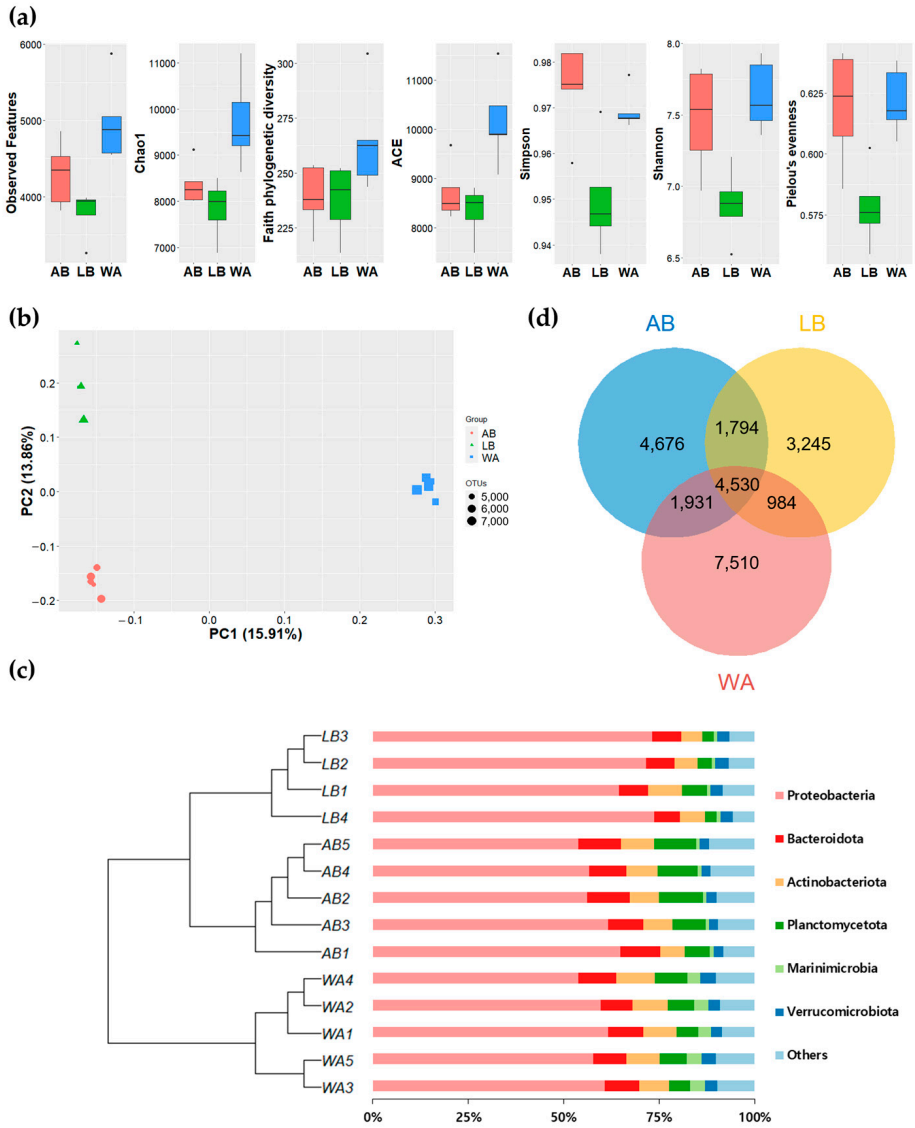

Figure 3. (**a**) Comparison of the alpha diversity values between three regions. WA showed the highest microbial diversity and generally high microbial evenness with AB. LB showed the lowest microbial diversity and evenness. (**b**) PCoA results based on Jaccard distance performed at OTU level of AB, LB, and WA. Three regions were separated from each other, but clustered into Korea and China groups. (**c**) UPGMA Tree based on Jaccard distance combined with microbial composition and relative abundance of top 10 phyla in AB, LB, and WA. (**d**) Venn diagram representing the distribution of OTU numbers among AB, LB, and WA.

3.4. Molecular Clustering Analysis of Seawater Microorganisms

The unweighted Pair Group Method with Arithmetic mean (UPGMA) tree was used to classify groups of similar samples based on the Jaccard similarity distance (Figure 3c). The tree shows that the seawater samples were largely grouped into two regions, Korea and China, with samples from China further subdivided into AB and LB. The distribution of the OTUs in each sample is represented by a Venn diagram (Figure 3d). Overall, 4676, 3245, and 7510 OTUs were specific to AB, LB, and WA, respectively. WA had the least overlap with the other two regions and exhibited a high number of OTUs unique to WA, indicating its distinct microbial composition. The number of common OTUs observed in all samples was 4530.

4. Discussion

4.1. Taxonomical Differences of Seawater Microbial Communities between Wando and Rongcheng

We analyzed microbial community assemblages in seawater from *S. japonica* aquaculture farms in Wando, Korea, and Rongcheng, China. Dissimilarities in the microbial composition were observed among the three sampling regions. At the phylum level, Proteobacteria, which were predominant in all our samples, are frequently detected on the surface of kelp and seaweeds [29–31]. Other microorganisms showed spatial specificity in abundance, such as Planctomycetota being the most abundant in China, especially in AB, while Marinimicrobia was the most abundant in WA. Planctomycetota is noteworthy for producing sulfatases, which can degrade sulfated polysaccharides called fucoidans, a major component of the kelp cell wall [32–34]. In addition, *Planctomycetes* are commonly found at the apex of algal tissues and are known to be involved in the degradation of older tissues [35]. The higher abundance of Planctomycetota in the Rongcheng region might be connected to the degradation of kelp tissue in the aquaculture farms in Rongcheng. Marinimicrobia has been reported to be capable of oxidizing hydroxylamine using the hao gene [36]. Hydroxylamine is an intermediate product of ammonia oxidation and is toxic to plants, animals, and even humans [37]. Accordingly, *S. japonica* in WA might have experienced less impact from the toxicity of hydroxylamine due to the abundance of Marinimicrobia. Therefore, it was anticipated that the abundance of specific bacteria involved in the nitrogen cycle in seawater could positively influence the growth of *S. japonica*.

At the family level, *Gimesiaceae*, which is more abundant in AB, is known for its association with freshwater inflows [38], indicating that the AB region may have previously been subjected to freshwater turbulence, probably via fluctuations in dissolved oxygen levels and salinity [39]. It is noteworthy that *Nitrincolaceae* often appears in increased abundance during the post-algal bloom season by actively utilizing biopolymeric materials derived from algae [40]. The higher abundance of *Nitrincolaceae* in LB may be linked to the concentration of decomposed polymeric material from unharvested or detached *S. japonica*, or due to plankton blooms, such as red tides. To identify the factors influencing the observed differences in microbial communities among these habitats, further research incorporating environmental variables and data from nearby kelp farms is necessary.

4.2. Microbial Biodiversity and Its Implications

In this study, the microbial diversity was lower in Rongcheng (both in AB and LB) than in Wando. The greater the microbial diversity in a specific region, the greater the adaptability or resistance to rapid environmental changes that may be possessed by the component organisms of the community in that region, including those induced by climate change [41]. Steiner et al. [42] also found evidence that marine communities, including highly diverse bacteria and algae, can enhance post-disturbance resilience. Diverse marine bacteria associated with macroalgae exert beneficial effects on their hosts [43]. Many marine bacteria provide hosts with a source of fixed nitrogen [44,45] or growth factors such as minerals and carbon dioxide [46,47]. The Wando region exhibited higher bacterial diversity and a lower abundance of pathogenic bacteria. Therefore, it can be assumed that kelp in aquaculture farms in Wando may have a greater chance of achieving adaptive benefits

from the more diverse microbial assemblage and the beneficial interrelationships that may emerge. Thus, this might be a possible explanation to the fact that kelp in Wando exhibited a more stable status while those in Rongcheng underwent a severe reduction in the 2022 harvesting season. To substantiate this speculation, additional research is needed on the physiological and ecological performance of *S. japonica* in relation to specific bacterial taxa that have the potential to benefit or harm the hosts.

Significant differences in microbial community composition among sampling regions were also observed. The PCoA scatter plot showed that the samples were largely clustered by country, without significant separation between the two sites (AB and LB) in Rongcheng. This means that samples with similar microbial communities were clustered closely together, indicating that there are distinctive differences in microbial communities by sampling region. This result was also consistent with the UPGMA tree and OTU Venn diagrams. Compared with AB and LB, WA showed a higher abundance of taxa such as *Thioglobaceae*, *Actinomarinaceae*, *Cryomorphaceae*, and *Pirellulaceae*, and a lower abundance of taxa such as *Rhodobacteraceae* and *Methylophilaceae*, and differences in these taxa may have contributed to the clustering separation. Meanwhile, Vieira et al. [48] reported lower bacterial diversity and abundance in the more polluted inner bay areas. Chinese sites, which showed lower microbial diversity in this study, may be affected by certain negative drivers compared with the WA region. One factor may be the difference in the human population in the two regions (approximately 700,000 in Rongcheng City vs. 50,000 in the Wando Islands). Ecophysiological parameters may also influence bacterial diversity, including connections between marine microorganisms and larger organisms, which are often observed in symbiosis and pathogenesis [49]. Therefore, the difference in bacterial diversity between Rongcheng and Wando, as shown in our study, provides an important preliminary explanation for the kelp production crisis in Rongcheng, and a stronger conclusion can be drawn with the accumulation of eDNA data coupled with specific microbe-kelp interaction studies.

4.3. Pathogenic Bacteria Related to Kelp Diseases

In this study, four genera, *Pseudoalteromonas*, *Vibrio*, *Pseudomonas*, and *Sulfitobacter,* were more abundant in the waters of Rongcheng than in Wando. Some species of these genera are associated with or cause diseases in kelps, including *S. japonica*. *Pseudoalteromonas* and *Vibrio* have been reported to cause the hole-rot disease in *S. japonica* [50]. Hole-rot disease is induced by alginase-secreting bacteria that decompose host cell walls, causing rotting and hole formation in the kelp blade [18]. *Pseudoalteromonas bacteriolytica* and *Pseudoalteromonas elyakovii* are known specifically to cause red spot disease on *S. japonica* [51,52]. Certain bacteria, including *Vibrio* and *Pseudomonas*, can use algal compounds such as laminaran, mannitol, and alginate as substrates, which can cause epidemic diseases in kelp [24]. In addition, *Pseudomonas* can be found frequently in *S. japonica* with green-rot disease and can turn the stipes of kelp sporelings to a greenish color, making them soft and decayed, eventually resulting in the leaves falling from the nursery ropes [53]. *Sulfitobacter* is an epiphytic bacterium commonly found in infected *S. japonica* tissues [16]. Furthermore, *Sulfitobacter* can produce polyhydroxyalkanoate (PHA) in a mannitol-abundant environment, but research regarding diseases of *S. japonica* caused directly by *Sulfitobacter* is scant. The significantly higher prevalence of these pathogenic bacteria in the seawater of Rongcheng, China, compared to Wando, Korea, suggests a strong correlation with mass mortality and the reduced harvest yield of cultivated kelp observed in Chinese waters in 2022.

This study serves as a meaningful starting point for subsequent studies that aim to identify causal factors of diseases in *S. japonica*. Nonetheless, the presence of these four pathogenic bacterial groups in Wando, despite the Wando kelp harvest in 2022 being unaffected, suggests that the difference in density within these groups (or any particular species) between the two regions may represent a critical threshold density per unit quantity of seawater that triggers the disease. Morris et al. [54] found that kelp from more polluted regions showed lower survival and growth, but these were restored when the abundance

of microbes was reduced. This study also revealed that an intermediate level of microbial abundance was optimal for kelp from unimpacted offshore areas, indicating that a specific level of microbial abundance can benefit kelp.

Although our results showed a significant difference in the abundance of pathogenic bacteria between two regions, there is a limitation in that this comparison is based on a snapshot of samples taken in winter. Nevertheless, this study suggests two points for the future perspective. First, identifying the threshold density of pathogenic bacterial groups or species in the seawater around the kelp aquafarm area will be necessary as a useful tool for early-warning systems in managing an intensive and heavily loaded aquaculture facility. Second, using eDNA metagenomic tool, we need to establish a regular and systematic monitoring system for the sea waters of large-scaled aquafarm areas such as Wando and Rongcheng. Creating a genetic database for kelp pathogenic bacteria or applying onsite eDNA analysis methods could enable the existing early-warning system even faster and more accurate for practical purpose.

5. Conclusions

Our results of metagenomic analysis using the microbial 16S rRNA region with datasets derived from eDNA extraction characterized the microbiome composition of the two *S. japonica* aquaculture systems as exhibiting high diversity and low abundance in the case of Wando, and low diversity and high abundance (for pathogenic groups) in the case of Rongcheng. The microbial composition of WA was distinct from that of the other two sites, AB and LB, which clustered together with higher similarity. In this study, we elucidated the differences in microbial community structures in seawater within kelp aquaculture farms and inferred the cause of decreased kelp productivity in the Rongcheng region through results indicating a high abundance of candidate pathogenic bacteria. Since there is only a limited amount of research on pathogenic bacteria related to kelp disease, and no critical pathogens have been definitively identified, this study is limited to presenting results for only candidate pathogenic bacteria. Therefore, it is anticipated that if physiological research on the relationship between kelp and pathogenic bacteria accumulates, we could establish biomarkers for the health management of kelp aquaculture farms using eDNA-based indicators based on these findings. This snapshot comparison with 2022 winter data for Wando and Rongcheng provides valuable information about the range of threshold density of pathogenic bacteria, which is beneficial for kelp farm management in both Yellow Sea-shared regions.

Supplementary Materials: The following supporting information can be downloaded from https://www.mdpi.com/article/10.3390/d16040245/s1. Table S1: Sampling information of seawater samples from Korea and China; Table S2: OTU and read number information of seawater samples; Table S3: Result of taxonomic analysis of each sample classified into OTUs from kingdom to species level (excel file); Figure S1: Rarefaction and extrapolation curves of OTUs at 97% similarity for each seawater sample from (a) AB, (b) LB, and (c) WA.

Author Contributions: Conceptualization: J.H.K.; formal analysis, investigation: S.C., K.M.Y., D.M.C., H.P. and J.H.K.; writing—original draft preparation: S.C., K.M.Y., H.P. and J.H.K.; field sampling, technical support: Y.H.C., X.W., L.W., X.L., D.D., H.P. and J.H.K.; supervision: H.P. and J.H.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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