Core bacteria associated with *Cochlodinium polykrikoides*(Dinophyta) blooms: diversity and function

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Abstract

Harmful algal blooms (HABs) of *Cochlodinium polykrikoides* exert pressure on nutritional resources; however, little is known about how they affect bacterial diversity. To investigate this, 110 water samples were collected from the Southern Sea of South Korea. Samples were divided into three groups based on environmental factors and phytoplankton data with a similarity of 85% using non-metric multidimensional scaling. Group I represented high-severity blooms and had a mean *C. polykrikoides* abundance of 1,560 cells mL^{-1}. Groups II and III represented low-severity blooms, with mean densities of 68 and 57 cells mL^{-1}, respectively. Inorganic nitrogen and phosphorous and dissolved organic carbon concentrations increased with *C. polykrikoides* density. This may reflect the change in biogeochemical cycling due to HAB release of extra polymeric substances. Furthermore, a total of 88 core bacterial operational taxonomic units (OTUs, with relative abundance > 1%) were identified. These included Gammaproteobacteria (36 OTUs), Flavobacteriia (24), Alphaproteobacteria (18), and other taxa (11). In Group I, the relative abundances of Gammaproteobacteria and Alphaproteobacteria were higher, and the relative abundance of Flavobacteriia was lower compared those in Groups II and III. Functional analysis based on the core bacterial OTUs revealed that chemoheterotrophy-related functions were more common in Group I than in Groups II and III. OTU #030, which was selected as strong indicator species, was strongly positive correlated with *C. polykrikoides* abundance (r = 0.95). Our results demonstrate that there are complex interactions between HABs, environmental factors, and core bacteria and functions, which could have important implications for biogeochemical cycling.

Introduction

Phytoplankton in marine ecosystems are an essential component of biogeochemical processes (Arrigo, 2005). Approximately 300 phytoplankton species form harmful algal bloom species (HABs); in particular, dinoflagellates cause red tides and many other widespread problems (Smayda, 1997). Moreover, many dinoflagellate species in HABs can produce toxins in marine ecosystems (Morse et al., 2018). Unarmoured HABs, such as *Cochlodinium polykrikoides* frequently bloom in many coastal countries (Matsuoka et al., 2010; Tang & Gobler, 2012). In South Korea, *C. polykrikoides* blooms have occurred frequently every year since 1995, causing serious economic losses such as fish kills (Jung et al., 2018; Kim et al., 2007). In particular, the southern coastal seas adjacent to the cities of Geoje, Tongyeong, and Yeosu in South Korea have registered the most severe *C. polykrikoides*blooms (Jung et al., 2018). The occurrence of HABs poses significant challenges to marine ecosystems as inorganic nutrients and dissolved organic matter change rapidly due to the biogeochemical interactions between HABs and bacterial communities. Kang et al. (2021) and Jung et al. (2021) reported the ecological interaction between unarmoured*Akashiwo sanguinea* HABs and specific bacterial populations, as well as the changes in biogeochemical cycling in coastal ecosystems due to this

interaction. However, ecological information on the changes in biogeochemical processes and bacterial communities is limited for *C. polykrikoides* HABs. Moreover, information on the interaction between bacterial communities and *C. polykrikoides* HABs (Jung et al., 2017) and the changes in the micro-ecosystem during blooms, including information on changes in environmental and microbial communities, is scarce, particularly regarding the changes in dissolved organic carbon (DOC) and inorganic nutrient concentrations and how to control the population dynamics of HAB organisms.

Interactions between phytoplankton and bacteria play significant roles in shaping biogeochemical cycles (Azam & Malfatti, 2007). This interconnection and co-influence between bacteria and phytoplankton (particularly the strong responses of HABs) is known as the phycosphere (Zhou et al., 2019). The marine phycosphere of specific bacteria and phytoplankton has precise cross-associations for the stimulation and inhibition of both groups (Sapp et al., 2007). The phytoplankton in the phycosphere supply carbohydrate sources to feed bacteria (Seymour et al., 2017), and, in turn, phytoplankton survive by utilising inorganic nutrients that are remineralised from organic matter by bacteria (Buchan et al., 2014; Worden et al., 2015). Thus, the relationships between bacteria and HABs are a promising study area in bacterial ecology, which may provide insights into how bacteria interact with HAB development (Andersson et al., 2010). Although some recent reports have suggested that specific bacteria are associated with HABs (Jung et al., 2021; Kang et al., 2021), ecological studies on these interactions are lacking. Moreover, the ecological interaction and function between bacteria and phytoplankton can be difficult to explain fully, because the phycosphere habitat may determine how communities are organised; for example, it can provide ecological niches where species interactions, such as competition, mutualism, and predation, occur, in addition to providing a suitable environment (Sapp et al., 2007; Zhou et al., 2019).

With advanced molecular technologies, several ecological studies have used metabarcoding approaches to understand the changes in microbial dynamics (Kang et al., 2021) and strengthen biological monitoring in the ocean (Jung et al., 2018). Our previous studies have revealed changes in the dynamics of bacterial communities in natural ecosystems (i.e., "what is there") (Kim et al., 2016) as well as in bacterial functional roles associated with phytoplankton dynamics (i.e., "why is it there") (Jung et al., 2021). In the present study, we explored the changes in the environmental characteristics and functional roles of core bacterial communities during *C. polykrikoides* HABs and estimated the core bacteria and environments in the phycosphere of *C. polykrikoides* HABs. In particular, to accurately estimate the ecological phenomena, we investigated large-scale (approximately 85 km × 20 km) *C. polykrikoides* blooms in the Southern Sea of South Korea using a bi-daily monitoring plan to understand the relationship between the core bacteria and *C. polykrikoides* HABs.

Materials and methods

Sample collection and environmental monitoring

The study sites were located in Geoje, Tongyeong, and Yeosu coastal waters approximately 85 km long in the Southern Sea of South Korea, in a generally shallow area with many islands (Fig. 1a). This survey area is subjected to a strong increase in inorganic nutrient sources originating from the food supply in fisheries (Jung et al., 2018). A total of 110 samples were collected bi-daily from 1 m below the sea surface at each sampling site between September 2 and 4, 2019. To simultaneously investigate the coastal areas of Geoje, Tongyeong, and Yeosu, three research teams and three ships performed the investigation. To account for the daily vertical migration of *C. polykrikoides* , the survey was conducted from 9 am to 5 pm. Dissolved oxygen (DO), pH, salinity, and water temperature were measured using YSI EXO2 Sonde probes (Yellow Springs, OH, USA). A volume of 5 L of seawater was collected from the surface layer, and each sub-sample was immediately prepared on the ship. The methodology for determining dissolved inorganic nutrients, DOC, and chlorophyll-*a* concentrations was described in our previous report (Kang et al., 2021) and is presented in the Supplementary Information. To avoid counting errors due to the destruction of unarmoured dinoflagellates, including *C. polykrikoides* , due to Lugol's fixation, two phytoplankton samples were collected in two 1-L acid-cleaned polyethylene bottles. One sample was immediately fixed with 5% Lugol's solution (Sigma, St. Louis, MO, USA) on the ship. The other sample was stored at 4 °C and transported to the laboratory

without any chemical fixation, which can cause cell destruction. Phytoplankton counting and identification methods are described in detail in the Supplementary Information. We obtained duplicated sub-samples to analyse all environmental and biological parameters.

Metabarcoding analyses of bacteria

Metabarcoding analysis of prokaryotic bacteria was performed following our previous methods (Jung et al., 2021; Kang et al., 2021). To remove large-sized inorganic and organic particles, each 500-mL seawater sample was pre-filtered using a 3-μm polycarbonate filter (TSTP04700; Millipore, Bedford, MA, USA). The bacterial communities were then harvested from the pre-filtered seawater using a 0.2 μm polycarbonate filter (GTTP04700). For bacterial metabarcoding, all samples were analysed in duplicate. Extraction of bacterial genomic DNA and metabarcoding analyses using 16S rDNA were performed as described in our previous report (Kang et al., 2021) and in the Supplementary Information (Table S1). Then, the amplified PCR products were analysed using a next-generation sequencing platform (Mi-Seq, Illumina, San Diego, CA, USA), and raw sequencing data (Fastq files) were analysed using the bioinformatics processes described in Jung et al. (2021) and in the Supplementary Information.

Statistical interpretation of the data

Results are presented as the means of the duplicate samples. Pearson's correlation was used to examine the relationships between the identified bacterial operational taxonomic units (OTUs), phytoplankton abundance, and environmental factors, using SPSS v.12 (SAS Institute Inc., Cary, NC, USA). An ordination plot was produced through non-metric multidimensional scaling (nMDS), according to Clarke (1993), using the ranked similarity matrix in PRIMER 6 v. 6.1.13. The detailed method is described in the Supplementary Information. Environmental and biological factors among groups were compared using one-way analysis of variance (ANOVA) using SPSS v.12, followed by Scheffe's post hoc test ($p < 0.05$). The alpha diversity (Shannon, Chao1, and Simpson indices) were plotted using R Studio (v. 1.2.5042) and a combination of the vegan (Oksanen et al., 2020), ape (Paradis et al., 2004), and ggplot (Wickham, 2016) packages. The analysis of indicator value (IndVal) was used to identify indicator in the most abundant bacterial OTUs (i.e., the core bacteria; each OTU displaying a relative abundance >1% in at least one sample) among the groups obtained by hierarchical agglomerative clustering analysis using the "indicspecies" function in R Studio (De Cáceres, 2013). The IndVal ranged from 0 (no an indicator species) to 1 (maximum indicator ability). Redundancy analysis (RDA) was subsequently used to investigate relationships between the IndVal OTUs and all factors, including phytoplankton and environmental factors, for the groups obtained from nMDS described previously. A functional annotation of prokaryotic taxa (FAPROTAX) analysis (Louca et al., 2016) was performed using the python script collapse_table.py (available at http://www.zoology.ubc.ca/louca/FAPROTAX) using the fourth-root-normalised OTU data. The functional composition in each module were calculated by multiplying the calculated values ("function tables") (Rivett & Bell, 2018).

Results

Changes in the environmental characteristics of *C. polykrikoides* HABs

The changes in the environmental characteristics of the Southern Sea of South Korea between September 2 and 4, 2019 are shown in Figure S1. *C. polykrikoides* blooms had more than 1,000 cells mL^{-1} in seven samples; the greatest abundance of *C. polykrikoides* (9,978 cells mL^{-1}) was observed in a sample (T-D-5) taken on September 4 (Fig. 1b and 1c). The abundance of *C. polykrikoides* was significantly correlated with pH, DO, dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP), DOC, and chlorophyll-*a* concentrations (Fig. 2). Other environmental factors (salinity and dissolved silica) were not significantly correlated with *C. polykrikoides* abundance (p > 0.05). The phytoplankton community, including *C. polykrikoides* and environmental factors, were classified into three groups at a similarity of 85% based on the results of nMDS analysis (Fig. 3a). Group I was associated with severe *C. polykrikoides* blooms focused on Yeosu coastal waters. In this group, the mean abundance of *C. polykrikoides* was 1,560 cells mL^{-1} in nine samples (Fig. 3a, Table 1). Groups II and III were associated with low severity to no *C. polykrikoides* blooms, with a mean abundance of 68 and 57 cells mL^{-1}, respectively. In the three groups classified using nMDS analysis,

all factors except DO were significantly different (Table 1). In particular, as the number of *C. polykrikoides* cells increased, the values of chlorophyll-*a* , pH, DOC, and DIN significantly increased (Fig. 2 and Table 1).

Core bacterial OTUs in *C. polykrikoides* blooms

The 16S rDNA metabarcoding results obtained from the bacterial community in the Southern Sea of South Korea are summarised in Table S2. The number of sequences and read counts generated from the 110 samples were 1,329,673–123,965,307 and 2,929–274,931, respectively. In particular, the values of total bacterial sequences, read counts, and Shannon and Simpson indices were significantly lower in Group I compared with the other groups (Fig. 3b, Table 1). Furthermore, a total of 88 core bacterial OTUs were identified (i.e., OTUs with a mean relative abundance >1% in at least one sample). A Venn diagram (Fig. 3c) showed that there were 34, 39, and 35 core bacterial OTUs in Groups I, II, and III, respectively. However, only eight of the core bacterial OTUs were unique to Group I. Interestingly, there were 18 OTUs in common between all three groups (16.7% of the core bacterial OTUs).

Most of the core bacterial OTUs belonged to Gammaproteobacteria (36 OTUs), Flavobacteriia (24), and Alphaproteobacteria (18); other core bacterial OTUs belonged to Acidimicrobiia (3), Actinobacteria (1), Balneolia (1), Betaproteobacteria (1), Epsilonproteobacteria (1), Saprospiria (2), and Cyanobacteria (2). In Group I, the relative abundances of Gammaproteobacteria (24%) and Alphaproteobacteria (25%) were significantly higher, while that of Flavobacteriia (13%) was significantly lower compared in the two groups (Fig. 3d and Table 1). In Group I, the dominant OTUs were #047, #017, #219, and #212, with each having a relative abundance of > 5% and an accumulated relative abundance of 41% (Fig. 4). Meanwhile, the dominant OTUs (relative abundance > 5%) in Group II were #047, #102, and #019, and #047, #002, #102, and #033 in Group III. OTUs #019 and #102 were common in Groups II and III (Fig. 4 and Table S3). OTUs #047 was common in all three groups.

Relationships among the core bacterial OTUs, *C. polykrikoides*, and environmental factors

Pearson's correlations between the abundance of *C. polykrikoides* and relative abundances of core bacterial OTUs provided insights into the stimulation and inhibition effects of *C. polykrikoides* (Table S4). The abundance of *C. polykrikoides* was negatively correlated with several core bacterial OTUs (#012, #015, #023, #024, #029, #033, and #059) but positively correlated with other OTUs (#003 and #030) even though relative abundances of these OTUs were < 1%. In particular, OTU #030 and *C. polykrikoides* showed almost identical changes (r = 0.95, p < 0.001, Fig. 5). Moreover, OTU #030 was significantly positively correlated with DO, pH, DIN, DIP, DOC, and chlorophyll-*a* values (Table S4). The core bacterial OTUs for analysing IndVal were classified into four groups at a similarity of 82% based on the results of cluster analysis (Fig. 6a). 39 indicator species were selected in core bacteria dataset (Table S5 and Fig. 6b). Group III and IV involving severe *C. polykrikoides* blooms were significantly selected to ten and four indicator species, respectively. Specifically, four OTUs (#017, #030, #035, and #037) in Group III and four OTUs (#081, #093, #219, and #421) in Group IV were observed to have strong values of > 0.8. Furthermore, RDA analysis was performed to estimate the relationships between bacteria and environmental factors, including common phytoplankton populations (Fig. 7 and Table S3). RDA analysis revealed 9 and 5 variables of environmental factors and phytoplankton community, respectively in 110 sampling sites, and 39 core bacterial OTUs that accounted for 62.0% and 12.7% of the variance explained along the first and the second axes, respectively (F-value: 30.34, p < 0.001). OTUs #020, #030, #037, #050, and #055 were strongly associated with DOC and *C. polykrikoides* values, while OTUs #212 was associated with the chlorophyll-a value. OTU #016 was associated with the Diatoms value. OTUs #178 and #184 were associated with DIN, and OTUs #013, #007, and #002 were associated with the DSi value.

Functional annotation of core bacteria during *C. polykrikoides* blooms

To estimate the potential functions of the bacterial community, the 88 core bacterial OTUs were analysed using FAPROTAX (Fig. 8). Based on a functional annotation dataset, cluster analysis showed that Groups I and II were very similar (88% similarity), while Groups I and III exhibited a lower similarity (75%). In Group I, the most abundant functions were chemoheterotrophy and aerobic chemoheterotrophy. Metabolic

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functions associated with chemoheterotrophy were dominant (74%). Other common functions of OTUs in Group I were aromatic compound degradation and hydrocarbon degradation, which accounted for 10% of the total composition. In Group II, the dominant functions were aerobic- and chemoheterotrophy functions, accounting for 67% of the total composition. In addition, aromatic compound degradation, iron respiration, dark oxidation, symbiont, and pathogens were present in Group II. In Group III, the functions related to chemoheterotrophy accounted for 57% of the total composition, which was lower than that of other groups, whereas functions related to nitrogen (nitrogen reduction, respiration, and denitrification) accounted for 40% of the composition, which was higher than in the other groups.

Discussion

HABs have become an important ecological issue in coastal waters worldwide (Anderson et al., 2008; Heisler et al., 2008). In coastal ecosystems, inorganic nutrient sources are important for the development of HABs (Anderson et al., 2002; Zhou et al., 2008). Our results indicate that *C. polykrikoides* not only exerts pressure on nutritional resources but is also composed of a core bacterial community during HABs. Our investigation showed that DIN and DIP concentrations increased when *C. polykrikoides* abundance increased (Fig. 2). In particular, DIP concentration was the highest at the site (T-D5-1-04) with the most severe *C. polykrikoides* HABs (9,978 cells mL^{-1}). This is probably the result of the extracellular polymeric substances (EPS) released by *C. polykrikoides* cells. Koch et al. (2014) reported that *C. polykrikoides* can release organic polymeric substances, and bacterial communities benefit from secreted organic carbon compounds. This means that high inorganic phosphorus and carbon from EPS are rapidly dissolved into the environment (Buchan et al., 2014; Gobler & Sanudo-Wilhelmy, 2003; Yang et al., 2016). In our previous study (Kang et al., 2021), DIP and DOC values immediately responded to HABs of the unarmoured dinoflagellate *A. sanguinea* . Many researchers are interested in nutrient uptake during HABs. For example, Koch et al. (2014) analysed the uptake preferences of various nitrogen sources by *C. polykrikoides* . However, there have been no previous studies on the increase of nutrient sources in *C. polykrikoides* blooms in the ecosystem. Interactions between phytoplankton and bacteria, and the subsequent promotion of specific bacterial populations, play important roles in shaping *C. polykrikoides* blooms (Azam & Malfatti, 2007; Cole, 1982; Sapp et al., 2007; Seymour et al., 2017). The supply of DIN from *C. polykrikoides* relies on bacteria to remineralise organic matter, and these remineralized nutrients can be used as a growth source for the HABs if there are no other factors controlling the *C. polykrikoides* blooms (Worden et al., 2015). Thus, the immediate environmental effect of *C. polykrikoides* blooms is that inorganic nutrient sources, particularly nitrogen and phosphorous sources, are supplied to the coastal ecosystem due to the release of EPS by the HABs. In particular, *C. polykrikoides* blooms markedly increased biological carbon export into the ocean, which is key for changing ecosystem dynamics.

Bacterial communities can respond to phytoplankton blooms (Pinhassi et al., 2004), and HABs are known to be closely associated with host-specific bacteria (Mayali et al., 2011). Generally, bacteria can promote the growth of HABs through the release of EPS (Jung et al., 2021; Kang et al., 2021) or inhibit HABs (Jung et al., 2008). Bacteria can utilize the EPS released by HABs and play important roles in biogeochemical cycling in oceans (Azam, 1998; Jung et al., 2021). Our results generally confirm previous reports (Grossart et al., 2005; Sapp et al., 2007), which found that phycospheres harbour host-specific bacteria based on the ability of bacteria to utilize different types of organic matter. Alphaproteobacteria are highly presented in Group I, this bacterial group is commonly detected in the southern coastal waters of South Korea (Kim et al., 2016). However, it is difficult to conclude that the specific bacteria community is related to the *C. polykrikoides* bloom. Meanwhile, the relative abundance of Gammaproteobacteria was high in Group I compared to other groups. It is known that *Roseobacter* sp. (Rhodobacteraceae: Gammaproteobacteria) increases during *C. polykrikoides* blooms (Park et al., 2017). Moreover, Gammaproteobacteria is highly associated with an increase in nutrient and DOC concentrations (Cluff et al., 2014; Kim et al., 2016). In our previous field study (Kang et al., 2021) and laboratory microcosm study (Jung et al., 2021) on *Akashiwo sanguinea* HABs, Gammaproteobacteria increased with HAB severity and was associated with DOC and inorganic nutrients. Thus, Gammaproteobacteria are one of the most active groups in the remineralisation of organic matter, particularly DOC released from living organisms. Moreover, this group is more likely to

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be affected by the strong supplementation of nutrients released from phytoplankton (Cluff et al., 2014; Kim et al., 2016).

Recently, FAPROTAX using 16S rDNA metabarcoding data has been developed as an effective tool for predicting metabolic and ecologically relevant functions of bacterial communities (Louca et al., 2016). In the present study, we determined that the functional profiles of the bacterial communities differed among groups. Chemoheterotrophy and aerobic chemoheterotrophy were strongly predicted in Group I. These functions are known to have a major impact on the occurrence of HABs (Amblard et al., 1992; Jung et al., 2021). In contrast, chemoheterotrophic metabolism functions were less common in Groups II and III, whereas functions associated with nitrogen and hydrocarbon sources were more common. These results are similar to the changes in bacterial function observed during and after *A. sanguinea* blooms; functions associated with chemoheterotrophy increased during *A. sanguinea* blooms, whereas nitrogen-related functions, such as nitrogen reduction, respiration, and denitrification, increased after the red-tide (Jung et al., 2021). Chun et al. (2019) reported that the functions of fermentation, nitrate reduction, hydrocarbon degradation, and aerobic ammonia oxidation increased as a result of the introduction of nitrogen sources. In our study, DOC concentrations were significantly higher in Group I samples, in which chemoheterotrophy-related functions were abundant. In a competition study with freshwater *Microcystis aeruginosa* , cyanobacterial HABs, and a chemoheterotrophic bacterium, *Candida utilis* , the HABs were controlled by the addition of glucose in order to maintain the activity of *C. utilis* (Kong et al., 2013). Therefore, the high abundance of chemoheterotrophy in Group I could be related with the high amounts of EPS likely released by *C. polykrikoides* blooms. Thus, the functions of the different bacterial populations were consistent with the environmental impacts; particularly, chemoheterotrophy was strongly associated with *C. polykrikoides* bloom density. Specific bacterial OTUs could affect the growth of *C. polykrikoides* HABs (Table S4), and their effect could differ depending on the environmental conditions. Park et al. (2015) reported that different bacterial taxa were dominant in different blooming stages of *C. polykrikoides* . It is also important to elucidate ecological interactions, such as competition, commensalism, and/or mutualism (Croft et al., 2005; Jung et al., 2008).

In the present study, ten bacterial OTUs were significantly correlated with changes in *C. polykrikoides* abundance; eight OTUs had a negative correlation and two OTUs had a positive correlation (Table S4). These specific bacterial OTUs may play inhibitory and stimulatory roles in the HAB phycosphere. Lebeau and Robert (2003) described the inhibitory relationship between diatoms and bacteria. In addition, Sun et al. (2020) reported that *Skeletonema* sp. and *Thalassiosira* sp. (diatoms) had an inhibitory association with common bacterial OTUs. Thus, the negative correlation between specific bacteria and *C. polykrikoides* may be associated with antagonism towards HAB growth, which is likely to influence the niche preferences of several species (Jung et al., 2017; Park et al., 2015). In our study, when *C. polykrikoides* bloomed (Group I), bacterial diversity was lower than in the non-bloom areas (Groups II and III). Similarly, Sun et al. (2020) reported that the diversity of the bacterial community decreased in diatom blooming areas. HABs can provide niches for specific bacterial populations; for example, polysaccharide substrates released by HABs can provide an advantage to opportunistic bacteria (Taylor et al., 2014). Moreover, it was consistent with OTUs that were highly correlated with *C. polykrikoides* abundances, and some significant indicator species (i.e., #030 and #219). In addition, the RDA analysis elucidated significant associations between *C. polykrikoides* blooms and these indicator OTUs (#030, #037, and #055). In particular, the rare taxon OTU #030, strongly significant indicator species in HABs, was closely correlated with changes in *C. polykrikoides* (r = 0.95, p < 0.001). The metabarcoding sequence of OTU #030 had a 96% similarity with *Francisella persica* (Gammaproteobacteria), which was found to cause fever in guinea pigs (Suitor Jr & Weiss, 1961) and human infection (Challacombe et al., 2017). Additionally, this bacterium is capable of synthesizing vitamin B (Gerhart et al., 2018), which is an important limiting factor for the development of *C. polykrikoides* blooms (Koch et al., 2014). Therefore, this bacterium could supply vitamin B to *C. polykrikoides* . Similarly, Cui et al. (2020) found that Rhodobacteraceae and Thaumarchaeota were associated with *C. polykrikoides* blooms. These bacterial populations have been reported to have a vitamin B_{12} synthesis pathway (Doxey et al., 2015; Sañudo-Wilhelmy et al., 2014). Moreover, the common taxa are thought to be associated with the major functions, whereas the functions of rare taxa are currently difficult to determine (Pedrós-Alió, 2006). Thus,

the occurrence of HABs highlighted the potential interactions between bacteria and HABs and affect not only bacterial community structure, but also its diversity. In conclusion, core bacteria may use the organic matter produced by the *C. polykrikoides* blooms, with different bacteria utilising specific EPS produced by the HAB species. Notably, specific core bacteria in the *C. polykrikoides* blooms may be related to changes in specific biogeochemical cycling due to EPS released from HABs. However, it was difficult to identify clearly, because we do not have direct evidences for the effects of core bacteria by EPS. Therefore, in further study, we will investigate bacterial activity by EPS released from HAB species and biogeochemical cycling using artificial ecosystem studies such as in-door microcosm and field mesocosms.

Data Accessibility

The raw sequencing data (Fastaq files) of 16S rDNA genes obtained from the Mi-Seq platform were deposited in the Sequence Read Archive database at NCBI under project number of PRJNA 776831.

Author contributions

Jung S.W. designed the research plan; Kim H.-J., Kim K., Jung K., Park J.S., Cha, H.G., Cho, S.H., and Kim H.S. performed the experiments and analyzed data; Kim H.-J., Kang D., Park J.S., Baek S.H., Lee T.K. and Lee C.Y. discussed the results; Kim H.-J. and Jung S.W. wrote the paper.

Ethics declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Competing interests: There are no conflicts of interests to declare.

Figure legends

Figure 1. Maps showing the sampling sites, depths, and distribution of *Cochlodinium polykrikoides* blooms. a) The study areas located in Geoje, Tongyeong, and Yeosu coastal waters approximately 85 km long in the Southern Sea of South Korea. A total of 110 samples were collected bi-daily from 1 m under the sea surface at each sampling site between September 2 and 4, 2019. b–c) The distribution of *C. plykrikoides* blooms on b) September 2 and c) September 4.

Figure 2. Significant Pearson's correlations between *Cochlodinium polykrikoides* blooms and environmental factors. Red areas correspond to the abundance of C. polykrikoides. The r value in each figure (upper right) indicates the Pearson's correlation coefficient between each environmental factor and *C. polykrikoides* abundance.

Figure 3. Distribution of operational taxonomic units (OTUs) among bacterial communities in water with different severities of *Cochlodinium polykrikoides* blooms collected from 110 samples. (a) Nonmetric multidimensional scaling (nMDS) plot by the Bray–Curtis dissimilarity method using environmental and phytoplankton datasets. All data were normalized by the fourth roots. (b) Violin plots, including box plots (median, minimum, and maximum) showing alpha diversity (Chao 1, Shannon diversity, and Simpson evenness) of bacterial communities in each sampling group. (c) Venn diagram showing the

shared and unique bacterial OTUs between sampling groups. (d) Stacked bar graph showing the relative abundance at the class level of the bacterial communities in each sample group. Sample groups were determined based on the nMDS.

Figure 4. Heatmap showing the mean relative abundance of core bacterial operational taxonomic units (OTUs) in each sampling group. OTUs were included as "core" if they had a mean relative abundance > 1% in at least one sample. Hierarchical agglomerative clustering used the group average of the most abundant bacterial OTUs obtained using the Bray–Curtis similarity method.

Figure 5. Pearson correlation between *Cochlodinium polykrikoides* and bacterial OTU #030. Coloured areas in the figure correspond to *C. polykrikoides* abundances. The black line corresponds to bacterial OTU #030 relative abundance. Inner graph shows a positive correlation (r = 0.952, p < 0.001).

Figure 6 . Heatmap showing the mean relative abundances of bacterial indicator species in each sampling group. (a) Hierarchical agglomerative clustering used the group average of the most abundant bacterial OTUs obtained using the Bray–Curtis similarity method. (b) Heatmap showing the mean relative abundances of Indicator species among Groups. The identified bacterial OTU numbers are as displayed in the Table S3 and Significant indicator value are displayed in the Table S5.

Figure 7. Redundancy analysis (RDA) of the indicator bacterial operational taxonomic units (OTUs) associated with environmental and phytoplankton factors. The identified indicator bacterial species were as displayed in Fig. 6b and Table S5. Four coloured star shapes indicated Group I-IV presented in Fig. 6a. The lengths and angles of the red arrows represent the correlations between the environmental and phytoplankton factors, as indicated, and the first two RDA axes.

Figure 8. Heatmap showing the metabolic functional annotation analysis (FAPROTAX) of the core bacterial operational taxonomic units (OTUs) in three groups. Groups I–III were obtained from the nMDS analysis presented in Fig. 3a. Each OTU had a mean relative abundance > 1% in at least one sample presented in Fig. 4. Heatmap displays the fourth-root-normalised data of the bacterial relative abundance.

Table 1. Changes in phytoplankton and bacterial communities and in environmental factors in the Southern Sea of South Korea. The three groups (Group I, II, and III) were obtained using nMDS analysis (see Figure 3a). Results were analysed using one-way ANOVA and Scheffe tests. Letters (A and B) indicate significant differences among groups.

Group	Group	Group I	Group II	Group II
Phyto-plankton	Diatoms (cells mL^{-1})	36 ± 30^A	24 ± 26^A	$1,380 \pm 9$
	Dinoflagellates (cells mL^{-1})	9 ± 5^A	24 ± 16^B	32 ± 21^B
	C. polykrikoides (cells mL^{-1})	$1,560 \pm 1,240^B$	68 ± 96^A	57 ± 128
	Other phytoplankton (cells mL^{-1})	1 ± 3^A	1 ± 5^A	6 ± 11^A
	Total phytoplankton (cells mL^{-1})	$1,605 \pm 1,220^B$	117 ± 93^A	$1,475 \pm 9$
	Chlorophyll-a (µg L^{-1})	34.6 ± 22.3^B	5.2 ± 6.2^A	7.2 ± 5.6
Environments	Water temperature (°C)	22.96 ± 0.44^{AB}	23.19 ± 0.86^B	22.11 ± 1
	Salinity	32.06 ± 0.19^A	32.20 ± 0.74^A	33.58 ± 0
	Dissolved oxygen (mg L^{-1})	6.85 ± 0.61^A	6.91 ± 0.56^A	$6.80 \pm 0.$
	pH	8.25 ± 0.07^B	8.22 ± 0.11^B	$8.06 \pm 0.$
	Dissolved organic carbon (mg L^{-1})	1.79 ± 0.74^B	0.85 ± 0.3^A	$0.84 \pm 0.$
	Dissolved inorganic nitrogen (µM)	24.45 ± 20.33^B	6.08 ± 4.5^A	$7.78 \pm 3.$
	Dissolved inorganic phosphorus (µM)	0.86 ± 0.89^A	0.58 ± 0.93^A	1.354 ± 1
	Dissolved silica (µM)	8.88 ± 2.68^A	8.41 ± 5.58^A	14.56 ± 6
Bacteria	Total sequence	$26,978,011 \pm 26,969,746^A$	$59,150,487 \pm 28,990,077^B$	$45,251,30$
	Read counts (<97% cut-off)	$21,748 \pm 19,815^A$	$48,545 \pm 23,217^B$	$36,283 \pm$

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Group	Group	Group I	Group II	Group II
	Operational taxonomic units	376 ± 142	563 ± 161	527 ± 28
	Acidimicrobiia (%)	4.64 ± 1.39[A]	8.37 ± 3.29[AB]	12.80 ± 7
	Alpha-proteobacteria (%)	25.09 ± 6.54[B]	23.25 ± 4.97[B]	18.14 ± 6
	Gamma-proteobacteria (%)	23.62 ± 5.92[B]	17.56 ± 5.57[A]	24.41 ± 4
	Flavobacteriia (%)	13.14 ± 2.03[A]	24.54 ± 5.44[B]	26.22 ± 6
	Chao1 index	515 ± 153	731 ± 191	705 ± 33
	Shannon index	5.42 ± 0.64[A]	5.75 ± 0.43[B]	5,61 ± 0.
	Simpson index	0.92 ± 0.05[A]	0.95 ± 0.02[B]	0.95 ± 0.

Data: Mean ± standard deviation, N.S.: not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

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a) Sampling sites & depths

b) *Cochlodinium polykrikoides* (02, Sep)

c) *Cochlodinium polykrikoides* (04, Sep)

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