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Illumina MiSeq Analysis and Comparison of Freshwater Microalgal Communities on Ulleungdo and Dokdo Islands

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Abstract

Ulleungdo and Dokdo are volcanic islands with an oceanic climate located off the eastern coast of South Korea. In the present study, we used barcoded Illumina MiSeq to analyze eukaryotic microalgal genera collected from Seonginbong, the highest peak on Ulleungdo, and from groundwater sites on Dongdo and Seodo Islands, which are part of Dokdo. Species richness was significantly greater in the Seonginbong samples than in the Dongdo and Seodo samples, with 834 operational taxonomic units (OTUs) identified from Seonginbong compared with 203 OTUs and 182 OTUs from Dongdo and Seodo, respectively. Taxonomic composition analysis was also used to identify the dominant microalgal phyla at each of the three sites, with Chlorophyta (green algae) the most abundant phyla on Seonginbong and Dongdo, and Bacillariophyta (diatoms) the most abundant on Seodo. These findings suggest that differences in the abundances of Chlorophyta and Bacillariophyta species in the Seonginbong, Dongdo, and Seodo samples are due to variations in species richness and freshwater resources at each sampling location. To the best of our knowledge, this is the first report to detail freshwater microalgal communities on Ulleungdo and Dokdo. As such, the number of species identified in the Seonginbong, Dongdo, and Seodo samples might be an indicator of the ecological differences among these sites and varying characteristics of their microbial communities. Information regarding the microalgal communities also provides a basis for understanding the ecological interactions between microalgae species and other eukaryotic microorganisms.

Key words: amplicon sequencing, Dokdo Island, microalgal community, MiSeq system, Ulleungdo Island

Introduction

Ulleungdo and Dokdo, located to the east of the Korean peninsula, are volcanic islands formed by the lava flows resulting from volcanic activity. Ulleungdo consists of one main island, with Seonginbong as its highest peak, and several small islets. Dokdo comprises two major islets, Dongdo and Seodo, and several exposed rocks (Sohn 1995; Kim et al. 2013). Ulleungdo and Dokdo share an oceanic climate due to the influence of warm and cold currents (Chang et al. 2002; Lee et al. 2010), although average annual precipitation is higher on Ulleungdo (1574 mm) than on Dokdo (660 mm). Annual average temperatures of both islands range from 12°C to 14°C (Chang et al. 2002; Lee et al. 2010). These islands are characterized by steep slopes that facilitate significant surface runoff when it rains, and it is thereby difficult for rainwater to collect on the surface. Indeed, volcanic islands formed from the lava are often characterized by a water-deficient environment. However, Ulleungdo and Dokdo have springs or small streams that originate from the groundwater to create an environment wherein fresh surface water is available (Sohn 1995; Chang et al. 2002).

The uneven distribution of freshwater sources influences the overall vegetation community and its successional processes. Ulleungdo, due to its relatively high precipitation, has greater vegetation species richness, with 487 vascular plants species and 104 woody plant species, than Dokdo, with 46 vascular plant species and eight woody plant species (Shin et al. 2004; Kim et al. 2007; Park et al. 2010), indicating that Ulleungdo is at

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a more advanced successional stage than Dokdo (Kim et al. 2007; Park et al. 2010; Jung et al. 2014). These patterns also extend to the microbial ecosystems, meaning that the different environments of Ulleungdo and Dokdo affect their microbial communities (Busse et al. 2006; Han et al. 2007; Djukic et al. 2010; Merilä et al. 2010). However, previous studies on the microbial communities on these islands have focused on the fungal and bacterial complements thereof (Kim et al. 2014; Nam et al. 2015), and little is known about the microalgal constituent. The discovery of new microalgal species is important in terms of the use of the algal biomass as a biological resource under different environmental conditions (Krustok et al. 2015).

Microalgae participate in carbon, nitrogen, and phosphorus cycles (Lehman 1980; Berner 1992; Vitousek et al. 2002) and, as photosynthetic organisms, are key producers and pioneers across a range of ecosystems (Booth 1941; Jackson 1971; Bellinzoni et al. 2003). In early successional stages, microalgae are the predominant production group, facilitating the subsequent arrival of herbaceous and woody plants, which can grow in the fertilized environment created by the microalgae (Booth 1941; Jackson 1971; Bellinzoni et al. 2003). The microalgal group promotes successional vegetation processes and allows for the emergence of predators and pathogenic microbes. The former mainly comprises zooplankton such as nematodes and arthropods (Havens and DeCosta 1987; Canovas et al. 1996; Mayer et al. 1997), while the latter causes disease in plants and animals and inhibits the biodegradation capacity of microbes (Littler and Littler 1998; Chen et al. 2014).

Interactions between microalgae and their abiotic and biotic environments drive the evolution of the microalgal community. Species dominance depends on environmental conditions, such as inorganic nutrient composition, water temperature, and light (Prowse and Talltng 1958; Goldman and Shapiro 1973; Porter 1977). In particular, microalgae composition is dominated by large-cell and needle-type algae, which are difficult to prey. Because the microalgal community supports the ecosystem and serves the producer-consumer relationship, analysis of this community can improve our understanding of the local environment, elemental recycling (carbon, nitrogen, and phosphorus), and micro-ecosystem relationships between producer and consumer trophic levels (Berner 1992; Vitousek et al. 2002; Cardinale et al. 2011). However, microalgal community research based solely on the culturing faces certain limitations, particularly the difficulty in identifying and analyzing unculturable microorganisms (Handelsman 2004; Streit and Schmitz 2004). Consequently, amplicon sequencing analysis using Illumina MiSeq can be a powerful tool for the investigation of unculturable microorganisms in their natural environment (Knight 2000; Handelsman 2004; Streit and Schmitz 2004; Schloss and Handelsman 2005).

Previous studies have yet to analyze the microalgal communities in the freshwater ecosystems on Ulleungdo (Seonginbong) and Dokdo (Dongdo and Seodo). This study investigated eukaryotic microalgal communities on these islands by taking freshwater samples from groundwater and tributary streams for the Illumina MiSeq analysis. Illumina MiSeq allows a large amount of sequencing information to be processed in a short time, and taxonomic analyses can then be conducted based on this information (Handelsman 2004; Streit and Schmitz 2004; Buée et al. 2009; Shokralla et al. 2012). In this study, microalgal species richness and diversity were characterized using taxonomic analysis, revealing that the composition of these communities varied by region, from phylum to species units.

Experimental

Materials and Methods

Collection of samples. Freshwater samples were collected from freshwater sources on Seonginbong (37° 30' 05.9" N 130° 52' 04.9" E) in Buk-myeon, Ulleung-gun, Gyeongsangbuk-do, South Korea, and on Dongdo (37° 14' 21.0" N 131° 52' 10.4" E) and Seodo Islands (37° 14' 31.5" N 131° 51' 51.6" E) in Dokdo-ri, Ulleung-gun, Gyeongsangbuk-do, South Korea (Supplemental Fig. S1). Seonginbong is the highest peak on Ulleungdo, and tributaries flow from here to freshwater sources. Freshwater sources are rare on Dokdo because of smaller volumes of groundwater, with only one groundwater source each on Dongdo and Seodo. Freshwater resources were harvested by collecting 100 ml from the water surfaces at each site on October 3, 2018. The collected samples were shipped to Macrogen Co., Ltd. on October 3, 2018, using the Same Day Express Courier Service and analyzed while maintained at room temperature.

DNA extraction and MiSeq system analysis. MiSeq system analysis (Macrogen, Seoul, South Korea) involved amplicon sequencing of whole DNA, with DNA extracted by the PowerSoil® DNA Isolation Kit (Cat. No. 12888, MO BIO) according to the manufacturer's protocol (Claassen et al. 2013). Extracted DNA was amplified with PCR to assess the 18S region for identifying eukaryotic microorganisms. Each sequenced sample was prepared according to the Illumina 18S MiSeq System Library protocols (Vo and Jedlicka 2014). DNA quantification and quality measurements were conducted using PicoGreen and Nanodrop. The 18S rRNA genes were amplified using 18S V4 primers (Stoeck et al. 2010; Luddington et al. 2012; Tragin et al. 2018). The amplicon PCR forward primer

sequence was TAReuk454FWD1 (5'-CCAGCA(G-C)C(C-T)GCGGTAATTCC-3'), and the amplicon PCR reverse primer sequence was TAReukREV3 (5'-ACTTTCGTTCTTGAT(C-T)(A-G)A-3') (Stoeck et al. 2010). Input gDNA was amplified using targeted DNA fragments (18S V4 primers size, 420 bp), and subsequent limited-cycle amplification was conducted to add multiplexing indices and Illumina sequencing adapters (Meyer and Kircher 2010). The final products were normalized and pooled using PicoGreen, and the sizes of the libraries were verified using the TapeStation DNA D1000 ScreenTape system (Agilent). The Illumina MiSeq data was analyzed on the MiSeq™ platform (Illumina, San Diego, USA; Kozich et al. 2013).

Taxonomic identification analysis. After sequencing, the Illumina MiSeq data were demultiplexed using the index sequence, and a FASTQ file was generated for each sample. The adapter sequence was removed using SeqPurge (Sturm et al. 2016), and error correction was performed on the overlapping areas of the two readings, with low-quality barcode sequences (read length < 400 bp or average quality value < 25) trimmed and filtered out. All raw Illumina MiSeq reads were identified using a BLASTN search of the NCBI database based on their barcode sequences (Zhang et al. 2000). If the results could not be taxonomically classified into a sublevel, unclassified (uc) was added to the end of the name. Operational taxonomic units (OTUs) were analyzed using CD-HIT at a 97% sequence similarity threshold (Unno et al. 2010; Li et al. 2012; Chen et al. 2013). The mothur platform was used to calculate rarefaction curves and diversity indices (Shannon, Simpson, and Chao1; Heck et al. 1975; Schloss et al. 2009). Beta diversity, which refers to sample diversity information among samples in a comparison group, was obtained based on weighted UniFrac distances. A UPGMA tree was used to visualize the flexibility between samples (FigTree, http://tree.bio.ed.ac.uk/software/figtree/) and demonstrate relationships among the three sites.

Results and Discussion

Sequencing results analysis. Table I presents the total number of reads and OTUs obtained from the three study sites. A total of 580 853 reads were sequenced from Seonginbong, with 290 919 validated reads remaining after preprocessing. The mean read length was 408.1 bp, and the maximum read length was 418 bp. A total of 534 141 reads were sequenced from Dongdo, with 289 610 validated reads remaining after preprocessing. The mean read length was 416.7 bp, and the maximum read length was 418 bp. A total of 469 920 reads were sequenced from Seodo, and the number of validated reads after preprocessing was 275 387. The mean read length was 412.54 bp, and the maximum read length was 418 bp. As seen in Table I, the Seonginbong sample contained the highest number of OTUs with 834 units, while the Dongdo and Seodo samples contained fewer OTUs at 203 and 182 units, respectively.

Table I
Illumina MiSeq results for the operational taxonomic units (OTUs) and statistical analysis.

	Seonginbong	Dongdo	Seodo
Total reads	580 853	534 141	469 920
Validated reads	290 919	289 610	275 387
Mean read length (bp)	408.1	416.7	412.54
Maximum read length (bp)	418	418	408
Number of OTUs[1]	834	203	182
Chao1[2]	834	203.75	182
Shannon[3]	6.722	2.038	5.118
Simpson[4]	0.9655	0.5569	0.9174
Goods Coverage[5]	1	0.9999	1

[1] OTUs: Operational taxonomic units
[2] Chao1: Species richness estimation
[3] Shannon: Shannon diversity index (> 0, higher is more diverse)
[4] Simpson: Simpson diversity index (0–1, 1 = most simple)
[5] Goods Coverage: 1 – (number of singleton OTUs/number of sequences); 1 = 100% coverage

The species richness of the samples is represented by rarefaction curves in Fig. 1, while the Chao1 species richness, the Shannon diversity index, and the Simpson diversity index are summarized in Table I (Heck et al. 1975; Schloss et al. 2009). The Seonginbong sample had the greatest species richness for all indicators (Chao1: 934; Shannon: 6.7222; Simpson: 0.9655), while Dongdo and Seodo had similar results to one another for Chao1 (203.75 and 182, respectively). However, Seodo had Shannon and Simpson index scores (5.118 and 0.9174, respectively) that were similar to those at Seonginbong (6.722 and 0.9655, respectively), and much higher than those of Dongdo (2.038 and 0.5569, respectively). Based on the OTU and species richness results, the diversity of the eukaryotic microbial composition on Seonginbong appeared to be greater than on Dongdo and Seodo (Fig. 1 and Table I). These results confirmed differences in species diversity among Seonginbong, Dongdo, and Seodo.

Analysis of the eukaryotic microbial communities on Seonginbong, Dongdo, and Seodo. After a BLASTN search of the NCBI database, the validated reads in Table I were assigned to a eukaryotic microbial taxonomic group (Table II; Niu et al. 2010). When a BLASTN search generated a specific scientific name with regards to phylum, class, order, family, genus, or species, the OTU was labeled as classified (c); if not, it was labeled as unclassified (uc). Table II summarizes the number of classified and unclassified OTUs

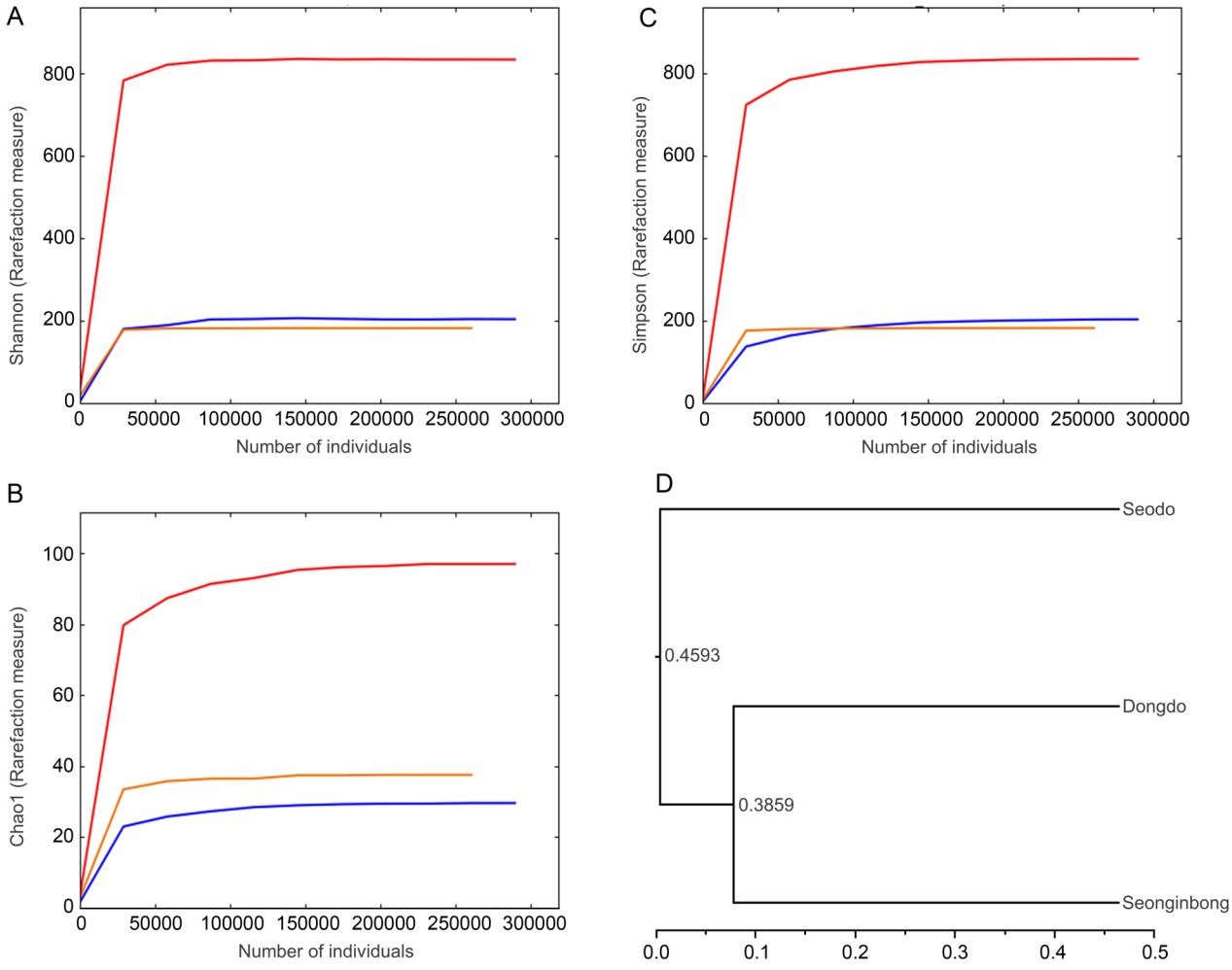

Fig. 1. Rarefaction curves for operational taxonomic units (OTUs) from the Seonginbong, Dongdo, and Seodo samples. (a) Shannon, (b) Simpson, and (c) Chao1 indexes. (d) UPGMA tree based on the community structures of Seonginbong, Dongdo, and Seodo. Seonginbong (red line), Dongdo (blue line), and Seodo (orange line).

from phylum to species for the Seonginbong, Dongdo, and Seodo samples. For the Seonginbong, Dongdo, and Seodo samples, 165 646, 30 911, and 164 678 reads were classified, and 125 273, 258 699, and 110 709 reads were unclassified at the phylum level, respectively. At the class level, 128 160, 23 011, and 144 662 reads were classified, and 162 759, 266 599, and 130 725 reads were unclassified for the Seonginbong Dongdo and Seodo regions respectively. In addition 99 964, 22 791, and 123 329 reads, respectively, were classified at the order

Table II
Number of eukaryotic microalgal taxa observed in the Seonginbong, Dongdo, and Seodo samples.

	Seonginbong		Dongdo		Seodo	
	c[1]	uc[2]	c[1]	uc[2]	c[1]	uc[2]
Phylum	165 646	125 273	30 911	258 699	164 678	110 709
Class	128 160	162 759	23 011	266 599	144 662	130 725
Order	99 964	190 955	22 791	266 819	123 329	152 058
Family	96 751	194 168	22 751	266 859	120 206	155 181
Genus	92 628	198 291	22 707	266 903	110 674	164 713
Species	84 154	206 765	17 930	271 680	97 541	177 846

[1]Number of sequencing reads with a scientific name for the taxon (classified, *c*)
[2]Number of sequencing reads either unclassified into a sublevel or classified as an unknown name for the taxon (unclassified, *uc*)

level. Similarly, 96 751, 22 751, and 120 206 reads were classified at the family level, and 92 628, 22 707, and 110 674 reads were classified at the genus level. Only 84 154, 17 930, and 97 541 sequences were classified at the species level. The number of validated reads was lower than the number of total reads because of the lack of information on unculturable microorganisms in the NCBI database. Therefore, the total reads and validated reads were both utilized for microorganism classification from the phylum to species level. Total reads and validated reads at the species level could be classified using information about their taxonomic levels, such as phylum, class, order, family, and genus.

The taxonomic compositions of the eukaryotic microbial communities on Seonginbong, Dongdo, and Seodo were then analyzed. It was found that the communities contained a combination of 17 phyla: Xanthophyceae, Streptophyta, Rotifera, Porifera, Platyhelminthes, Nematoda, Eustigmatophyceae, Chytridiomycota, Chordata, Chlorophyta, Blastocladiomycota, Basidiomycota, Bacillariophyta, Ascomycota, Arthropoda, Apicomplexa, and Annelida (Fig. 2). The communities were dominated by the microalgal phyla Chlorophyta and Bacillariophyta, although their combined relative abundance was significantly higher in the Dongdo and Seodo samples (93.52% and 91.77%, respectively) than in the Seonginbong sample (31.02%). Differences in population densities were more profound in the Seonginbong sample than in the Dongdo and Seodo samples (Fig. 2). This analysis of differences in the community composition could contribute significantly to our understanding of the microbial ecosystems at each site (Wegley et al. 2007; Rodriguez-Brito et al. 2010; Fierer et al. 2012). Microbial community compositions already reported suggest a need for further research on the eukaryotic microorganisms in each region (Knight 2000; Chiao 2004; Schloss and Handelsman 2005). In this regard, amplicon sequencing using Illumina MiSeq is a powerful tool for the identification of unculturable microalgae. More important, MiSeq system analysis can also generate useful information on new species in the natural environments of Ulleungdo and Dokdo that could be helpful in studying unculturable eukaryotic microorganisms.

Comparison of the microalgal communities on Seonginbong, Dongdo, and Seodo. We compared the structures of the microalgal communities on Seonginbong, Dongdo, and Seodo by constructing phylogenetic trees (Fig. 1) using UPGMA analysis with eukaryotic microorganisms. The taxonomic compositions of Seonginbong, Dongdo, and Seodo were analyzed from the phylum to species level. Overall, it was found that Seonginbong was more closely related to Dongdo than Seodo. At the phylum level, the microalgal communities of Seonginbong, Dongdo, and Seodo exhibited differences in their taxonomic compositions despite being dominated by two phyla: Chlorophyta (Round 1963) and Bacillariophyta (Fig. 2; Kaczmarska et al. 2007). The relative abundance of Chlorophyta was very high on Dongdo (93.52%), while Bacillariophyta was dominant on Seodo (89.13%). On Seonginbong, the relative abundance of Chlorophyta was higher than that of Bacillariophyta (Chytridiomycota 39.43%, Chlorophyta 27.1%, and Bacillariophyta 4.31%).

At the class level, five distinct microalgal classes (Bacillariophyceae, Coscinodiscophyceae, Chlorophyceae, Trebouxiophyceae, and Ulvophyceae) were detected in the overall sample (Fig. 3), with the dominant groups in each region differing: Seonginbong, Chlorophyceae; Dongdo, Trebouxiophyceae; and Seodo, Bacillariophyceae. In particular, the relative abundance of Bacillariophyceae was higher in Seodo (88.24%) than

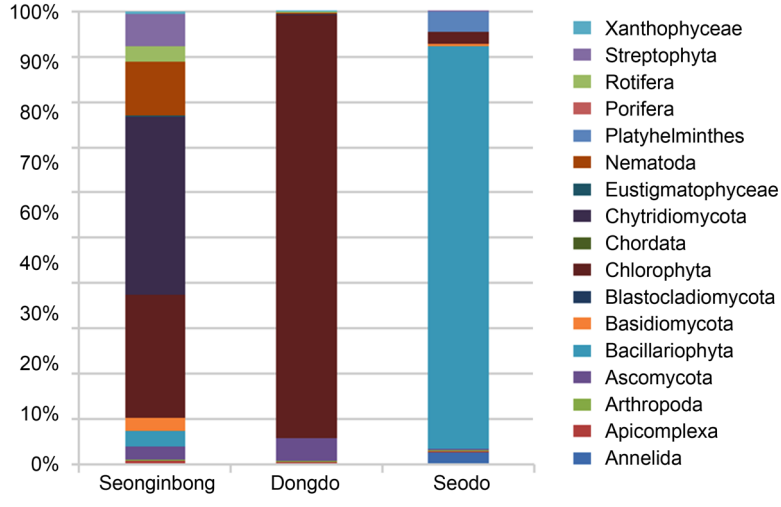

Fig. 2. Taxonomic composition of the eukaryotic microbial phyla on Seonginbong, Dongdo, and Seodo.

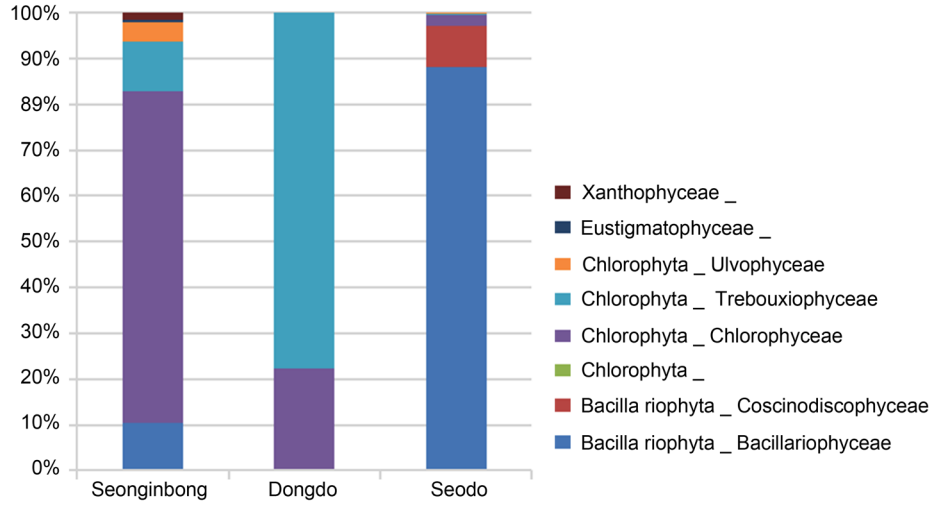

Fig. 3. Taxonomic composition of the microalgal classes on Seonginbong, Dongdo, and Seodo.

in Seonginbong (10.56%) or Dongdo (0%). The Coscinodiscophyceae was only present on Seodo (8.86%). In addition, two or three green algae classes were present at the study sites, including Chlorophyceae (73.39%), Trebouxiophyceae (11.17%), and Ulvophyceae (4.18%) on Seonginbong; Chlorophyceae (22.22%) and Trebouxiophyceae (77.67%) on Dongdo; and Chlorophyceae (2.28%), Trebouxiophyceae (0.49%), and Ulvophyceae (0.13%) on Seodo.

A total of 30 families were detected in each region. Seventeen families had identified scientific names, and nine had a relative abundance of at least 1%. These families are summarized in Table III. On Seonginbong, three diatom families (*Bacillariaceae*, *Pinnulariaceae*, and *Stauroneidaceae*) and eight green algae families (*Characiochloridaceae, Chlamydomonadaceae, Chlorococcaceae, Scenedesmaceae, Coccomyxaceae, Chlorellaceae*, and *Ctenocladaceae*) were identified, with the most dominant being *Chlorococcaceae* (1.53%), and two unclassified green algae families (*Chlorophyta, Chlorophyceae, Chlamydomonadales*: 3.47%; *Chlorophyta, Chlorophyceae, Sphaeropleales*: 2.53%). Conversely, only one diatom or green algae family was dominant in Dongdo and Seodo. One diatom family (*Diadesmidaceae*) and three green algae families (*Chlamydomonadaceae, Chlorococcaceae*, and *Chlorellaceae*) were present on Dongdo, with the most dominant being *Chlorellaceae* (64.91%), distantly followed by *Chlorococcaceae* (18.46%). Conversely, Seodo had nine diatom families (*Achnanthaceae, Bacillariaceae, Amphipleuraceae, Diadesmidaceae, Naviculaceae, Sellaphoraceae, Catenulaceae*, and *Stephanopyxidaceae*) and two green algae families (*Scenedesmaceae* and *Chlorellaceae*). The dominant family on Seodo was an unclassified diatom family (21.62%), distantly followed by three other diatom families (*Bacillariaceae*: 3.26%, *Sellaphoraceae*: 3.12%, and *Stephanopyxidaceae*: 3.14%) with relative abundances of at least 3%. Four families were found to be unique to a specific area: *Stauroneidaceae* on Seonginbong and *Achnanthaceae*, *Sellaphoraceae*, and *Stephanopyxidaceae* on Seodo. In summary, although Dongdo and Seodo are proximally located, the species composition on Seodo differs from that on Seonginbong and Dongdo; these two regions exhibit greater similarity to one another than either does to Seodo.

A total of 50 microalgal genera were detected, with 37 identified by scientific name. Fourteen genera had a relative abundance of at least 1% (Table ???). Three diatom genera (*Nitzschia, Pinnularia*, and *Amphora*) known to produce toxins were identified on Seonginbong (*Pinnularia*, 0.09%) and Seodo (*Nizschia*, 3.26%; *Amphora*, 0.12%). For the diatom genera with a relative abundance of at least 1%, genera were uniquely distributed in each region; however, microalgal genera were found at all three sites. In particular, the microalgal taxonomic compositions of Seonginbong and Dongdo were more similar to one another than either was to Seodo. There were six dominant genera (*Stauroneis*, 1.16%; *Chlorococcum*, 1.53%; *Chlorosarcinopsis*, 1.29%; *Bracteacoccus*, 1.89%), and two unclassified microalgal genera (1.48% and 1.47%) present on Seonginbong. On Dongdo, unclassified microalgal genera (63.78%), *Chlorococcum* (18.46%), and *Pseudochlorella* (1.13%) dominated. Six diatom genera were dominant on Seodo (*Achnanthidium*, 20.76%; *Achnanthes*, 1.54%; *Nitzschia*, 3.26%; *Diadesmis*, 2.15%; *Sellaphora*, 3.12%; *Stephanopyxis*, 3.14%). These findings indicate that microalgal genera are widely distributed across all three regions, whereas diatom genera are restricted to specific areas. Of note, microalgal taxonomic composition showed that the Seonginbong and Dongdo communities were closely related at the genus level.

For species-level analyses, the microalgal species identified from the Seonginbong, Dongdo, and Seodo

Table III
Relative abundance of eukaryotic microalgal families in the Seonginbong, Dongdo, and Seodo samples.

Taxonomy				Seonginbong		Dongdo		Seodo	
Phylum	Class	Order	Family	%[1]	Fr[2]	%[1]	Fr[2]	%[1]	Fr[2]
Bacillariophyta	Bacillariophyceae	–	–	0.00	0	0.00	0	21.62	59 531
Bacillariophyta	Bacillariophyceae	–	Achnanthaceae	0.00	0	0.00	0	1.54	4 239
Bacillariophyta	Bacillariophyceae	–	Bacillariaceae	0.15	423	0.00	0	3.26	8 974
Bacillariophyta	Bacillariophyceae	Naviculales	Amphipleuraceae	0.00	0	0.00	0	0.23	645
Bacillariophyta	Bacillariophyceae	Naviculales	Diadesmidaceae	0.00	0	0.00	7	2.38	6 551
Bacillariophyta	Bacillariophyceae	Naviculales	Naviculaceae	0.00	0	0.00	0	0.29	802
Bacillariophyta	Bacillariophyceae	Naviculales	Pinnulariaceae	0.09	256	0.00	0	0.00	0
Bacillariophyta	Bacillariophyceae	Naviculales	Sellaphoraceae	0.00	0	0.00	0	3.12	8 579
Bacillariophyta	Bacillariophyceae	Naviculales	Stauroneidaceae	1.16	3 379	0.00	0	0.00	0
Bacillariophyta	Bacillariophyceae	Thalassiophysales	Catenulaceae	0.00	0	0.00	0	0.12	329
Bacillariophyta	Coscinodiscophyceae	Melosirales	Stephanopyxidaceae	0.00	0	0.00	0	3.14	8 660
Bacillariophyta	Coscinodiscophyceae	Paraliales	–	0.00	0	0.00	0	0.13	354
Chlorophyta	–	–	–	0.00	0	0.02	65	0.00	0
Chlorophyta	–	Chlorodendrales	–	0.00	0	0.01	21	0.00	0
Chlorophyta	Chlorophyceae	–	–	0.03	91	0.00	13	0.62	1 708
Chlorophyta	Chlorophyceae	Chlamydomonadales	–	2.53	7 368	0.09	271	0.00	0
Chlorophyta	Chlorophyceae	Chlamydomonadales	Characiochloridaceae	0.34	1 002	0.00	0	0.00	0
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlamydomonadaceae	0.38	1 096	0.02	48	0.00	0
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlorococcaceae	1.53	4 437	18.46	53 463	0.00	0
Chlorophyta	Chlorophyceae	Chlorosarcinales	–	1.29	3 761	0.00	0	0.00	0
Chlorophyta	Chlorophyceae	Sphaeropleales	–	3.47	10 096	0.00	2	0.00	0
Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	0.08	243	0.00	0	0.22	597
Chlorophyta	Trebouxiophyceae	–	–	0.56	1 624	0.00	0	0.12	329
Chlorophyta	Trebouxiophyceae	–	Coccomyxaceae	0.01	23	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	Chlorellales	–	0.00	0	0.00	7	0.00	0
Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	0.58	1 689	64.91	187 999	0.06	162
Chlorophyta	Trebouxiophyceae	Ctenocladales	Ctenocladaceae	0.21	604	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	Microthamniales	–	0.11	323	0.02	48	0.00	0
Chlorophyta	Ulvophyceae	Ulotrichales	–	0.55	1 605	0.00	0	0.00	0
Chlorophyta	Ulvophyceae	Ulvales	–	0.00	0	0.00	0	0.05	136

The microalgal families detected in at least one of the three samples are shown. Unclassified taxonomic names (phylum, class, order, and family) are replaced with a dash (–)
[1] Relative abundance
[2] Frequency of microalgae detected at each sampling site

samples were organized in a phylogenetic tree (Fig. 4). For groups without a scientific name at the genus level (Fig. 3), names were only added to those with scientific names at the species level (Fig. 4). Phylum and class boundaries were identified for the microalgal species based on species-level sequencing analysis for Seonginbong, Dongdo, and Seodo. In Fig. 4, the boundary between Bacillariophyta and Chlorophyta is marked with a yellow box, and boundaries between the classes belonging to each phylum are marked with purple boxes (Metting 1996). Among the microalgal groups, some of the Chlorophyceae belonged to Trebouxiophyceae from class via phylum (Tables III and IV). At the species level, dominant species were identified on each island, to include six species on Seonginbong, two species on Dongdo, and six species on Seodo; these are marked by boxes in Fig. 4 (Seonginbong, red; Dongdo, blue; Seodo, green). Of the species shown on the phylogenetic tree, some have been associated with shellfish toxins (Falconer 2012) frequently found on Seodo. In particular, *Nitzschia* sp. (Bates et al. 1989; Martin et al. 1990), known to be associated with shellfish toxins, was one of the dominant species on Seodo.

We organized the three microalgal communities from the phylum to species levels to analyze the taxonomic compositions of the three study sites. The approximate

Table IV
Relative abundance of eukaryotic microalgal genera in the Seonginbong, Dongdo, and Seodo samples.

Taxonomy					Seonginbong		Dongdo		Seodo	
Phylum	Class	Order	Family	Genus	%[1]	Fr[2]	%[1]	Fr[2]	%[1]	Fr[2]
Bacillariophyta	Bacillariophyceae	–	–	–	0.00	0	0.00	0	0.86	2 363
Bacillariophyta	Bacillariophyceae	–	–	*Achnanthidium*	0.00	0	0.00	0	20.76	57 168
Bacillariophyta	Bacillariophyceae	–	Achnanthaceae	*Achnanthes*	0.00	0	0.00	0	1.54	4 239
Bacillariophyta	Bacillariophyceae	–	Bacillariaceae	*Hantzschia*	0.15	423	0.00	0	0.00	0
Bacillariophyta	Bacillariophyceae	–	Bacillariaceae	*Nitzschia*	0.00	0	0.00	0	3.26	8 974
Bacillariophyta	Bacillariophyceae	Naviculales	Amphipleuraceae	–	0.00	0	0.00	0	0.23	645
Bacillariophyta	Bacillariophyceae	Naviculales	Diadesmidaceae	*Diadesmis*	0.00	0	0.00	0	2.15	5 922
Bacillariophyta	Bacillariophyceae	Naviculales	Diadesmidaceae	*Luticola*	0.00	0	0.00	7	0.23	629
Bacillariophyta	Bacillariophyceae	Naviculales	Naviculaceae	*Navicula*	0.00	0	0.00	0	0.29	802
Bacillariophyta	Bacillariophyceae	Naviculales	Pinnulariaceae	*Pinnularia*	0.09	256	0.00	0	0.00	0
Bacillariophyta	Bacillariophyceae	Naviculales	Sellaphoraceae	*Sellaphora*	0.00	0	0.00	0	3.12	8 579
Bacillariophyta	Bacillariophyceae	Naviculales	Stauroneidaceae	*Stauroneis*	1.16	3 379	0.00	0	0.00	0
Bacillariophyta	Bacillariophyceae	Thalassiophysales	Catenulaceae	*Amphora*	0.00	0	0.00	0	0.12	329
Bacillariophyta	Coscinodiscophyceae	Melosirales	Stephanopyxidaceae	*Stephanopyxis*	0.00	0	0.00	0	3.14	8 660
Bacillariophyta	Coscinodiscophyceae	Paraliales	–	*Paralia*	0.00	0	0.00	0	0.13	354
Chlorophyta	–	–	–	–	0.00	0	0.02	65	0.00	0
Chlorophyta	–	Chlorodendrales	–	–	0.00	0	0.01	21	0.00	0
Chlorophyta	Chlorophyceae	–	–	–	0.03	91	0.00	13	0.62	1 708
Chlorophyta	Chlorophyceae	Chlamydomonadales	–	–	1.48	4 308	0.09	249	0.00	0
Chlorophyta	Chlorophyceae	Chlamydomonadales	–	*Actinochloris*	0.00	0	0.01	22	0.00	0
Chlorophyta	Chlorophyceae	Chlamydomonadales	–	*Ettlia*	0.62	1 793	0.00	0	0.00	0
Chlorophyta	Chlorophyceae	Chlamydomonadales	–	*Spongiochloris*	0.44	1 267	0.00	0	0.00	0
Chlorophyta	Chlorophyceae	Chlamydomonadales	Characiochloridaceae	*Characiochloris*	0.34	1 002	0.00	0	0.00	0
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlamydomonadaceae	–	0.02	50	0.00	0	0.00	0
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlamydomonadaceae	*Chlamydomonas*	0.35	1 026	0.00	0	0.00	0

The microalgal genera detected in at least one of the three samples are shown. Unclassified taxonomic names (phylum, class, order, family, and genus) are replaced with a dash (–)
[1] Relative abundance
[2] Frequency of microalgae detected at each sampling site

Table IV
Continued.

Taxonomy					Seonginbong		Dongdo		Seodo	
Phylum	Class	Order	Family	Genus	%[1]	Fr[2]	%[1]	Fr[2]	%[1]	Fr[2]
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlamydomonadaceae	*Chloromonas*	0.01	20	0.02	48	0.00	0
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlorococcaceae	*Chlorococcum*	1.53	4 437	18.46	53 463	0.00	0
Chlorophyta	Chlorophyceae	Chlorosarcinales	–	*Chlorosarcinopsis*	1.29	3 761	0.00	0	0.00	0
Chlorophyta	Chlorophyceae	Sphaeropleales	–	–	1.47	4 271	0.00	2	0.00	0
Chlorophyta	Chlorophyceae	Sphaeropleales	–	*Bracteacoccus*	1.89	5 490	0.00	0	0.00	0
Chlorophyta	Chlorophyceae	Sphaeropleales	–	*Dictyochloris*	0.12	335	0.00	0	0.00	0
Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	*Coelastrella*	0.02	64	0.00	0	0.22	597
Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	*Desmodesmus*	0.06	179	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	–	–	–	0.04	102	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	–	–	*Myrmecia*	0.51	1 487	0.00	0	0.12	329
Chlorophyta	Trebouxiophyceae	–	–	*Watanabea*	0.01	35	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	–	Coccomyxaceae	*Coccomyxa*	0.01	23	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	Chlorellales	–	–	0.00	0	0.00	7	0.00	0
Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	–	0.06	182	63.78	184 700	0.06	162
Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	*Auxenochlorella*	0.34	999	0.00	2	0.00	0
Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	*Chlorella*	0.04	102	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	*Heveochlorella*	0.00	0	0.00	12	0.00	0
Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	*Lobosphaera*	0.00	14	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	*Pseudochlorella*	0.13	392	1.13	3 285	0.00	0
Chlorophyta	Trebouxiophyceae	Ctenocladales	Ctenocladaceae	*Leptosira*	0.21	604	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	Microthamniales	–	–	0.09	272	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	Microthamniales	–	*Dictyochloropsis*	0.02	51	0.00	0	0.00	0
Chlorophyta	Trebouxiophyceae	Microthamniales	–	*Stichococcus*	0.00	0	0.02	48	0.00	0
Chlorophyta	Ulvophyceae	Ulotrichales	–	–	0.55	1 605	0.00	0	0.00	0
Chlorophyta	Ulvophyceae	Ulvales	–	–	0.00	0	0.00	0	0.05	136

The microalgal genera detected in at least one of the three samples are shown. Unclassified taxonomic names (phylum, class, order, family, and genus) are replaced with a dash (–)
[1] Relative abundance
[2] Frequency of microalgae detected at each sampling site

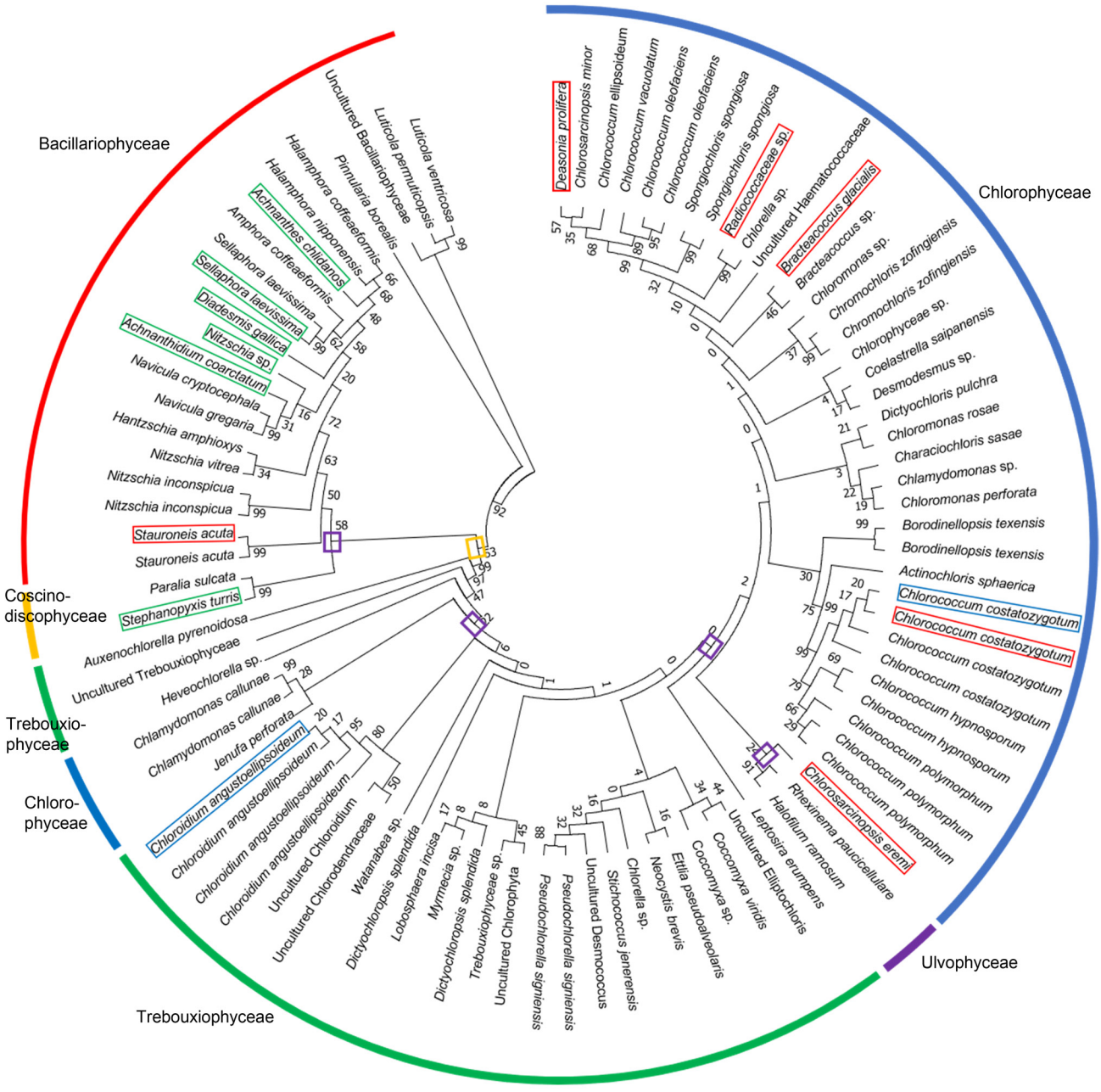

Fig. 4. Molecular phylogenetic analysis using a maximum likelihood (ML) tree. The boundary between phyla is marked with a yellow box, and the boundaries between classes are marked with purple boxes. Five classes are displayed about the species names in the phylogenetic tree. The dominant species in each sample is marked with a colored box (Seonginbong: red; Dongdo: blue; Seodo: green). The class of each group is presented at the edge (Bacillariophyceae; red, Coscinodiscophyceae; yellow, Chlorophyceae; blue, Trebouxiophyceae; green and Ulvophyceae; purple).

amount of available sunlight was highest at the Seonginbong sampling site and lowest at the Seodo site (Supplementary Fig. S1), and the relative abundance of diatoms strongly correlated with sunlight availability (Hudon and Bourget 1983; Post et al. 1984; Lange et al. 2011). Our results and those from previous studies indicate that further research on the relationship between light and microalgal community composition is required. Research also suggests that microalgal community composition is influenced by natural enemies or disease (Hudon and Bourget 1983; Post et al. 1984; Lange et al. 2011). In accordance with these findings, we observed differences in natural compositions among Seonginbong, Dongdo, and Seodo; the microalgal group was dominant on Seodo. At the phylum level, Seonginbong was characterized by zooplankton and pathogenic fungal groups (Fig. 2). At the class level, the microalgal group was dominated by Chlorophyceae on Seonginbong and Trebouxiophyceae (particularly *Chlorellaceae*) on Dongdo (Fig. 3). Trebouxiophyceae, which contains a family of small-celled organisms (*Chlorellaceae*), are relatively vulnerable to predators compared to other

classes, and results of the present study suggest that the presence of consumers (zooplankton and pathogenic fungi) affects the dominance of Chlorophyceae on Seonginbong and Dongdo to a greater extent than on Seodo (Fig. 2 and 3; Johnson and Agrawal 2003; Sarma et al. 2003; Yoshida et al. 2004; Pradeep et al. 2015).

Previous studies also indicate that microalgae can affect the external environment. A previous report found that the *Trebouxia* genus of the Trebouxiophyceae class forms a symbiotic association with lichen, fungi, and algae and is directly involved in changes to the terrestrial environment (Ahmadjian, 1988; Piercey-Normore 2006). The results of this study indicated that Trebouxiophyceae was not accurately detected at the phylum level, although a greater presence of Trebouxiophyceae at the class level was found on Seonginbong than on Dongdo and Seodo, as evidenced by the identification of microalgal communities via eukaryotic microbial communities (Table III and IV). This suggests that the microalgal group on Seonginbong engages in a symbiotic relationship with the fungi group, unlike on Dongdo and Seodo, and that this relationship directly impacts the Seonginbong natural environment. Previous studies have found that microalgae secrete a range of substances that influence their natural environment, including fungal toxins and predators (Havens and DeCosta 1987; Canovas et al. 1996; Mayer et al. 1997; Falconer 2012). The genera *Nitzschia* (Bates et al. 1989; Martin et al. 1990), *Amphora* (Daniel et al. 1980), and *Paralia* (Sar et al. 2012) are reported to be closely associated with shellfish toxins on Seodo (Falconer 2012; Sar et al. 2012) that can be harmful to human health when ingested orally. Although they only account for a small fraction of the detected microalgal community, it is nonetheless necessary to monitor their toxin-producing abilities and biological resources. Our findings indicate that microalgae are influenced both by environmental factors and the surrounding microbial community and that characteristics of the microbial community are influenced by the natural environment.

Conclusion

The present study analyzed the overall species richness and taxonomic compositions of the microalgal communities of Ulleungdo (Seonginbong) and Dokdo (Dongdo and Seodo). Amplicon sequencing analysis was performed using Illumina MiSeq, and microbiological OTUs from Seonginbong (834), Dongdo (203), and Seodo (182) were identified. Three indicators (Chao1, Shannon, and Simpson) were used to analyze species richness, and it was found that the species richness of Seonginbong was higher than those of Dongdo and Seodo. Classified reads were used for taxonomic analysis, with the communities exhibiting differences in their composition from the phylum to species levels. In the Seonginbong sample, several other eukaryotic microorganisms were present in the community in addition to microalgae, while microalgae (Chlorophyta) and diatoms (Bacillariophyta) were found to be extremely dominant on Dongdo and Seodo, respectively. Analyses of the relative abundances of the different communities added details to information regarding the differences in species richness between the three regions. We obtained information on microalgae on Seonginbong, Dongdo, and Seodo via MiSeq tools; however, MiSeq analysis does have some limitations with regards to dependence on existing taxonomies in screening and identifying microalgal species. Despite these experimental limitations, MiSeq analysis provided in-depth information on the microalgae communities of Ulleungdo and Dokdo.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Ahmadjian V. The lichen alga *Trebouxia*: does it occur free-living? Plant Syst Evol. 1987;158(2-4):243–247. https://doi.org/10.1007/BF00936348

Bates SS, Bird CJ, Freitas ASW, Foxall R, Gilgan M, Hanic LA, Johnson GR, McCulloch AW, Odense P, Pocklington R, et al. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. Can J Fish Aquat Sci. 1989 Jul;46(7):1203–1215. https://doi.org/10.1139/f89-156

Bellinzoni AM, Caneva G, Ricci S. Ecological trends in travertine colonisation by pioneer algae and plant communities. Int Biodeterior Biodegradation. 2003 Apr;51(3):203–210. https://doi.org/10.1016/S0964-8305(02)00172-5

Berner RA. Weathering, plants, and the long-term carbon cycle. Geochim Cosmochim Acta. 1992 Aug;56(8):3225–3231. https://doi.org/10.1016/0016-7037(92)90300-8

Booth WE. Algae as pioneers in plant succession and their importance in erosion control. Ecology. 1941 Jan;22(1):38–46. https://doi.org/10.2307/1930007

Buée M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol. 2009 Oct;184(2):449–456. https://doi.org/10.1111/j.1469-8137.2009.03003.x

Busse MD, Beattie SE, Powers RF, Sanchez FG, Tiarks AE. Microbial community responses in forest mineral soil to compaction, organic matter removal, and vegetation control. Can J For Res. 2006 Mar;36(3):577–588. https://doi.org/10.1139/x05-294

Canovas S, Picot B, Casellas C, Zulkifi H, Dubois A, Bontoux J. Seasonal development of phytoplankton and zooplankton in a high-rate algal pond. Water Sci Technol. 1996 Mar;33(7):199–206. https://doi.org/10.2166/wst.1996.0139

Cardinale BJ, Matulich KL, Hooper DU, Byrnes JE, Duffy E, Gamfeldt L, Balvanera P, O'Connor MI, Gonzalez A. The functional role of producer diversity in ecosystems. Am J Bot. 2011 Mar;98(3):572–592. https://doi.org/10.3732/ajb.1000364

Chang KI, Kim YB, Suk MS, Byun SK. Hydrography around Dokdo. Ocean Polar Res. 2002 Dec 31;24(4):369–389. https://doi.org/10.4217/OPR.2002.24.4.369

Chen W, Zhang CK, Cheng Y, Zhang S, Zhao H. A comparison of methods for clustering 16S rRNA sequences into OTUs. PLoS One. 2013 Aug 13;8(8):e70837. https://doi.org/10.1371/journal.pone.0070837

Chen Z, Lei X, Zhang B, Yang L, Zhang H, Zhang J, Li Y, Zheng W, Tian Y, Liu J, et al. First report of *Pseudobodo* sp., a new pathogen for a potential energy-producing algae: *Chlorella vulgaris* cultures. PLoS One. 2014 Mar 5;9(3):e89571. https://doi.org/10.1371/journal.pone.0089571

Chiao JS, Sheng WG, Cheng XB. [An important mission for microbiologists in the new century-cultivation of the unculturable microorganisms]. Sheng Wu Gong Cheng Xue Bao. 2004 Sep;20(5):641–645.

Claassen S, du Toit E, Kaba M, Moodley C, Zar HJ, Nicol MP. A comparison of the efficiency of five different commercial DNA extraction kits for extraction of DNA from faecal samples. J Microbiol Methods. 2013 Aug;94(2):103–110. https://doi.org/10.1016/j.mimet.2013.05.008

Daniel GF, Chamberlain AHL, Jones EBG. Ultrastructural observations on the marine fouling diatom *Amphora*. Helgol Meeresunters. 1980 Jun;34(2):123–149. https://doi.org/10.1007/BF01984035

Djukic I, Zehetner F, Mentler A, Gerzabek MH. Microbial community composition and activity in different Alpine vegetation zones. Soil Biol Biochem. 2010 Feb;42(2):155–161. https://doi.org/10.1016/j.soilbio.2009.10.006

Falconer IR. Algal Toxins in Seafood and Drinking Water. London (UK): Academic Press; 2012.

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J. 2012 May;6(5):1007–1017. https://doi.org/10.1038/ismej.2011.159

Goldman JC, Shapiro J. Letter: Carbon dioxide and pH: effect on species succession of algae. Science. 1973 Oct 19;182(4109):306–307. https://doi.org/10.1126/science.182.4109.306

Han X, Wang R, Liu J, Wang M, Zhou J, Guo W. Effects of vegetation type on soil microbial community structure and catabolic diversity assessed by polyphasic methods in North China. J Environ Sci (China). 2007 Jan;19(10):1228–1234. https://doi.org/10.1016/S1001-0742(07)60200-9

Handelsman J. Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev. 2004 Dec 01;68(4):669–685. https://doi.org/10.1128/MMBR.68.4.669-685.2004

Havens K, DeCosta J. Freshwater plankton community succession during experimental acidification. Arch. Hydrobiol. 1987;111:37–65.

Heck KL Jr, van Belle G, Simberloff D. Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. Ecology. 1975 Oct;56(6):1459–1461. https://doi.org/10.2307/1934716

Hudon C, Bourget E. The effect of light on the vertical structure of epibenthic diatom communities. Bot Mar. 1983;26(7):317–330. https://doi.org/10.1515/botm.1983.26.7.317

Jackson TA. Study of the ecology of pioneer lichens, mosses, and algae on recent Hawaiian lava flows. Pac Sci. 1971;25:22–32.

Johnson MTJ, Agrawal AA. The ecological play of predator-prey dynamics in an evolutionary theatre. Trends Ecol Evol. 2003 Nov;18(11):549–551. https://doi.org/10.1016/j.tree.2003.09.001

Jung SY, Byun JG, Park SH, Oh SH, Yang JC, Jang JW, Chang KS, Lee YM. The study of distribution characteristics of vascular and naturalized plants in Dokdo, South Korea. J Asia-Pac Biodivers. 2014 Jun;7(2):e197–e205. https://doi.org/10.1016/j.japb.2014.03.011

Kaczmarska I, Reid C, Moniz M. Diatom taxonomy: morphology, molecules and barcodes. Paper presented at: Proceedings of the 1st Central-European Diatom meeting 2007: Botanic Garden and Botanical Museum Berlin-Dahlem FU-Berlin; 2007. p. 69–72.

Kim CH, Park JW, Lee MH, Park CH. Detailed bathymetry and submarine terraces in the coastal area of the Dokdo volcano in the Ulleung Basin, the East Sea (Sea of Japan). J Coast Res. 2013 Jan 02;65:523–528. https://doi.org/10.2112/SI65-089.1

Kim MH, Oh YJ, Kim CS, Han MS, Lee JT, Na YE. The flora and vegetation distribution in Dokdo. Korean J Environ Agric. 2007 Mar 27;26(1):85–93. https://doi.org/10.5338/KJEA.2007.26.1.085

Kim YE, Yoon H, Kim M, Nam YJ, Kim H, Seo Y, Lee GM, Ja Kim Y, Kong WS, Kim JG, et al. Metagenomic analysis of bacterial communities on Dokdo Island. J Gen Appl Microbiol. 2014;60(2):65–74. https://doi.org/10.2323/jgam.60.65

Knight IT. Molecular genetic methods for detection and identification of viable but nonculturable microorganisms. In: Colwell RR, Grimes DJ, editors. Nonculturable Microorganisms in the Environment. Boston: (MA, USA): Springer; 2000. p. 77–85. https://doi.org/10.1007/978-1-4757-0271-2_6

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol. 2013 Sep 01;79(17):5112–5120. https://doi.org/10.1128/AEM.01043-13

Krustok I, Truu J, Odlare M, Truu M, Ligi T, Tiirik K, Nehrenheim E. Effect of lake water on algal biomass and microbial community structure in municipal wastewater-based lab-scale photobioreactors. Appl Microbiol Biotechnol. 2015 Aug;99(15):6537–6549. https://doi.org/10.1007/s00253-015-6580-7

Lange K, Liess A, Piggott JJ, Townsend CR, Matthaei CD. Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. Freshw Biol. 2011 Feb;56(2):264–278. https://doi.org/10.1111/j.1365-2427.2010.02492.x

Lee YG, Kim BJ, Park GU, Ahn BY. Characteristics of precipitation and temperature at Ulleung-do and Dok-do, Korea for recent four years (2005~2008). J. Environ. Sci. Int. 2010 Sep 30;19(9):1109–1118. https://doi.org/10.5322/JES.2010.19.9.1109

Lehman JT. Release and cycling of nutrients between planktonic algae and herbivores. Limnol Oceanogr. 1980 Jul;25(4):620–632. https://doi.org/10.4319/lo.1980.25.4.0620

Li W, Fu L, Niu B, Wu S, Wooley J. Ultrafast clustering algorithms for metagenomic sequence analysis. Brief Bioinform. 2012 Nov 01;13(6):656–668. https://doi.org/10.1093/bib/bbs035

Littler MM, Littler DS. An undescribed fungal pathogen of reef-forming crustose corraline algae discovered in American Samoa. Coral Reefs. 1998 Jul 7;17(2):144. https://doi.org/10.1007/s003380050108

Luddington IA, Kaczmarska I, Lovejoy C. Distance and character-based evaluation of the V4 region of the 18S rRNA gene for the identification of diatoms (Bacillariophyceae). PLoS One. 2012 Sep 21;7(9):e45664. https://doi.org/10.1371/journal.pone.0045664

Martin JL, Haya K, Burridge LE, Wildish DJ. *Nitzschia pseudodelicatissima* – a source of domoic acid in the Bay of Fundy,

eastern Canada. Mar Ecol Prog Ser. 1990;67:177–182. https://doi.org/10.3354/meps067177

Mayer J, Dokulil MT, Salbrechter M, Berger M, Posch T, Pfister G, Kirschner AK, Velimirov B, Steitz A, Ulbricht T. Seasonal successions and trophic relations between phytoplankton, zooplankton, ciliate and bacteria in a hypertrophic shallow lake in Vienna, Austria. Hydrobiologia. 1997;342:165–174. https://doi.org/10.1023/A:1017098131238

Merilä P, Malmivaara-Lämsä M, Spetz P, Stark S, Vierikko K, Derome J, Fritze H. Soil organic matter quality as a link between microbial community structure and vegetation composition along a successional gradient in a boreal forest. Appl Soil Ecol. 2010 Oct;46(2):259–267. https://doi.org/10.1016/j.apsoil.2010.08.003

Metting F. Biodiversity and application of microalgae. J Ind Microbiol. 1996;17:477–489.

Meyer M, Kircher M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harb Protoc. 2010 Jun 01;2010(6):pdb.prot5448. https://doi.org/10.1101/pdb.prot5448

Nam YJ, Kim H, Lee JH, Yoon H, Kim JG. Metagenomic analysis of soil fungal communities on Ulleungdo and Dokdo Islands. J Gen Appl Microbiol. 2015;61(3):67–74. https://doi.org/10.2323/jgam.61.67

Niu B, Fu L, Sun S, Li W. Artificial and natural duplicates in pyrosequencing reads of metagenomic data. BMC Bioinformatics. 2010;11(1):187. https://doi.org/10.1186/1471-2105-11-187

Park SJ, Song IG, Park SJ, Lim DO. [The flora and vegetation of Dokdo Island in Ulleung-gun, Gyeongsanbuk-do]. Korean J Environ Ecol. 2010;24(3):264–278.

Piercey-Normore MD. The lichen-forming ascomycete *Evernia mesomorpha* associates with multiple genotypes of *Trebouxia jamesii*. New Phytol. 2006 Jan;169(2):331–344. https://doi.org/10.1111/j.1469-8137.2005.01576.x

Porter KG. The plant-animal interface in freshwater ecosystems: microscopic grazers feed differentially on planktonic algae and can influence their community structure and succession in ways that are analogous to the effects of herbivores on terrestrial plant communities. Am Sci. 1977;65:159–170.

Post AF, Dubinsky Z, Wyman K, Falkowski PG. Kinetics of light-intensity adaptation in a marine planktonic diatom. Mar Biol. 1984;83(3):231–238. https://doi.org/10.1007/BF00397454

Pradeep V, Van Ginkel S, Park S, Igou T, Yi C, Fu H, Johnston R, Snell T, Chen Y. Use of copper to selectively inhibit *Brachionus calyciflorus* (Predator) growth in *Chlorella kessleri* (Prey) mass cultures for algae biodiesel production. Int J Mol Sci. 2015 Aug 31;16(9):20674–20684. https://doi.org/10.3390/ijms160920674

Prowse GA, Talltng JF. The seasonal growth and succession of plankton algae in the White Nile. Limnol Oceanogr. 1958 Apr;3(2):222–238. https://doi.org/10.4319/lo.1958.3.2.0222

Rodriguez-Brito B, Li L, Wegley L, Furlan M, Angly F, Breitbart M, Buchanan J, Desnues C, Dinsdale E, Edwards R, et al. Viral and microbial community dynamics in four aquatic environments. ISME J. 2010 Jun;4(6):739–751. https://doi.org/10.1038/ismej.2010.1

Round FE. The taxonomy of the Chlorophyta. Brit Phycol Bull. 1963 Dec;2(4):224–235. https://doi.org/10.1080/00071616300650061

Sar EA, Sunesen I, Goya AB, Lavigne AS, Tapia E, García C, Lagos N. First report of diarrheic shellfish toxins in mollusks from Buenos Aires province (Argentina) associated with *Dinophysis* spp.: evidence of okadaic acid, dinophysistoxin-1 and their acyl-derivatives. Bol Soc Argent Bot. 2012;47:5–14.

Sarma SSS, Trujillo-Hernández HE, Nandini S. Population growth of herbivorous rotifers and their predator (*Asplanchna*) on urban wastewaters. Aquat Ecol. 2003;37(3):243–250. https://doi.org/10.1023/A:1025896703470

Schloss PD, Handelsman J. Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. Genome Biol. 2005;6(8):229. https://doi.org/10.1186/gb-2005-6-8-229

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009 Dec 01;75(23):7537–7541. https://doi.org/10.1128/AEM.01541-09

Shin H, Park S, Kang K, Yoo J. The establishment of conservation area and conservation strategy in Ulnung Island. Korean J Environ Ecol. 2004;18:221–230.

Shokralla S, Spall JL, Gibson JF, Hajibabaei M. Next-generation sequencing technologies for environmental DNA research. Mol Ecol. 2012 Apr;21(8):1794–1805. https://doi.org/10.1111/j.1365-294X.2012.05538.x

Sohn YK. Geology of Tok Island, Korea: eruptive and depositional processes of a shoaling to emergent island volcano. Bull Volcanol. 1995 Feb;56(8):660–674. https://doi.org/10.1007/BF00301469

Stoeck T, Bass D, Nebel M, Christen R, Jones MDM, Breiner HW, Richards TA. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol. 2010 Mar;19 Suppl 1:21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x

Streit WR, Schmitz RA. Metagenomics – the key to the uncultured microbes. Curr Opin Microbiol. 2004 Oct;7(5):492–498. https://doi.org/10.1016/j.mib.2004.08.002

Sturm M, Schroeder C, Bauer P. SeqPurge: highly-sensitive adapter trimming for paired-end NGS data. BMC Bioinformatics. 2016 Dec;17(1):208. https://doi.org/10.1186/s12859-016-1069-7

Tragin M, Zingone A, Vaulot D. Comparison of coastal phytoplankton composition estimated from the V4 and V9 regions of the 18S rRNA gene with a focus on photosynthetic groups and especially Chlorophyta. Environ Microbiol. 2018 Feb;20(2):506–520. https://doi.org/10.1111/1462-2920.13952

Unno T, Jang J, Han D, Kim JH, Sadowsky MJ, Kim OS, Chun J, Hur HG. Use of barcoded pyrosequencing and shared OTUs to determine sources of fecal bacteria in watersheds. Environ Sci Technol. 2010 Oct 15;44(20):7777–7782. https://doi.org/10.1021/es101500z

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB. Towards an ecological understanding of biological nitrogen fixation. In: Boyer EW, Howarth RW, editors. The nitrogen cycle at regional to global scales. Dordrecht (Germany): Springer; 2002. p. 1–45. https://doi.org/10.1007/978-94-017-3405-9_1

Vo ATE, Jedlicka JA. Protocols for metagenomic DNA extraction and Illumina amplicon library preparation for faecal and swab samples. Mol Ecol Resour. 2014 Nov;14(6):1183–1197. https://doi.org/10.1111/1755-0998.12269

Wegley L, Edwards R, Rodriguez-Brito B, Liu H, Rohwer F. Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. Environ Microbiol. 2007 Nov;9(11):2707–2719. https://doi.org/10.1111/j.1462-2920.2007.01383.x

Yoshida T, Hairston NG Jr, Ellner SP. Evolutionary trade-off between defence against grazing and competitive ability in a simple unicellular alga, *Chlorella vulgaris*. Proc R Soc Lond B Biol Sci. 2004 Sep 22;271(1551):1947–1953. https://doi.org/10.1098/rspb.2004.2818

Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. J Comput Biol. 2000 Feb;7(1-2):203–214. https://doi.org/10.1089/10665270050081478

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