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Temporal and spatial dynamics of phytoplankton diversity in the East China Sea near Jeju Island (Korea): A pyrosequencing-based study

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ABSTRACT

The East China Sea (ECS) has long been considered an important monitoring site for oceanic ecosystem changes because many water currents and river discharges constantly influence this area. In this study, the community structure and diversity of phytoplankton in the northern part of the ECS adjacent to Jeju Island were explored using small subunit ribosomal RNA (SSU) pyrosequencing. We analysed samples collected from four stations from the surface and at 30-m and 50-m depths during April and September 2011. We observed spatial and temporal variations in the phytoplankton community. Among phytoplankton, diatoms and dinoflagellates constituted a major portion at all stations (60–90%). However, comparison of the April and September samples showed seasonal variation and shifts in the dispersion of diatom and dinoflagellates among stations. Among stations, diatoms dominated St. 1 and others were dominated by dinoflagellates. Furthermore, phylotypes of potentially toxin-producing genera such as *Karlodinium*, *Heterocapsa*, *Gymnodinium*, *Gyrodinium*, and *Pseudo-nitzschia* were dominant in this area.

1. Introduction

Phytoplankton are primary producers that contribute approximately 50% of global primary production (Falkowski and Raven, 2013). They play a pivotal role in marine food webs and contribute significantly to global carbon cycling (Fehling et al., 2012). Phytoplankton are greatly influenced by various environmental factors and pollutants, and are thus often considered bioindicators of ecosystem health (McCormick and Cairns Jr, 1994; Rakocevic-Nedovic and Hollert, 2005). Furthermore, continuous monitoring of phytoplankton is important for the control of toxic algal blooms, which affect the surrounding biodiversity and disrupt ecosystem functions (Anderson et al., 2002). In addition, recent climate changes could affect ocean ice cover, temperature, precipitation, and circulation, and could lead to drastic changes in the community structure, composition, and productivity of phytoplankton (Sarmiento et al., 2004).

High-throughput sequencing has been widely used in metagenomic studies of micro- and pico-eukaryotic protist diversity targeting the hypervariable regions of the small subunit ribosomal RNA (SSU) (Faria et al., 2014; Boopathi et al., 2015). In

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addition, various other methods, including light microscopy flow cytometry and molecular techniques, such as terminal restriction fragment length polymorphism, microarray, real-time PCR, and SSU genes have been used to identify phytoplankton (Medlin et al., 2006). However, the use of high-throughput parallel pyrosequencing of SSU from environmental DNA allows rapid analysis of microbial communities (Zhan et al., 2013). High-throughput pyrosequencing methods have many advantages over morphological identification, including higher resolution, faster analysis, and the possibility of automation. Next-Generation Sequencing (NGS)-based approaches also facilitate the precise identification of rare and fragile phytoplankton and detect nano-and pico-phytoplankton (Faria et al., 2014).

The East China Sea (ECS), which is one of the largest continental shelves worldwide, is located on the western side of the North Pacific Ocean. The Yangtze River is the largest river that flows into the ECS, and significantly influences nutrient fluxes by virtue of Yangtze River diluted water (YRDW), which in turn influences the phytoplankton (Shi et al., 2014). Several dynamic currents including the Kuroshio Current (KC) and the Tsushima Warm Current (TWC) (Ichikawa and Beardsley, 2002) are known to influence the ECS, thereby creating various habitats with different nutrient regimes (Gong et al., 2003). Jeju Island is situated in the temperate zone, surrounded by the northern part of the ECS. In recent decades, harmful algal blooms (HABs) have created serious problems in the ECS region by affecting the marine environment and fisheries (Wang and Wu, 2009). Additionally, changes in climatic conditions influence environmental stressors, which induce drastic changes in functional phytoplankton groups and indicator species. Hence, this region of the ECS is considered an important monitoring site for changes in the ecosystem. Studies on phytoplankton in the ECS began in the late 1980s with the use of epifluorescence microscopy combined with flow cytometry (Vaulot and Xiuren, 1988). Many studies were targeted to specific taxonomic groups, such as diatoms, dinoflagellates, and cyanobacteria; however, only a few have covered the entirety of the eukaryotic phytoplankton communities in the northern part of the ECS. In a previous study, we determined the diversity of phytoplankton, including pico- and nano-sized organisms, around Jeju Island during spring using pyrosequencing analysis (Faria et al., 2014). Distinct distribution patterns of phytoplankton were observed among the surveyed sites (south-eastern [green algae dominant], south-western [dinoflagellate dominant], north-eastern [green algae dominant], and north-western [diatom dominant] regions of Jeju Island). The oceanic currents in the ECS might have caused the different patterns; however, dominant phytoplankton taxa and patterns may differ among seasons. Further, studies pertaining to seasonal variation in eukaryotic phytoplankton in the region are scarce, and available studies are based on microscopic and HPLC methods.

In the present study, we analysed phytoplankton communities and dynamics for spatial and temporal variation. We determined phytoplankton community structure based on samples taken in September from four stations along a transect between Jeju Island and the YRDW. We then compared the results with those from samples taken in April. In addition, we instituted a detailed comparison of diatoms and dinoflagellates, as these two groups were predominant among phytoplankton.

2. Materials and methods

2.1. Sampling stations and collection of samples

A field cruise (i.e., a training ship, A-Ra Ho, from the Jeju National University, Korea) was taken in the northern ECS and the coastal waters around Jeju Island from April 24–30, 2011. The sampling stations lie between Jeju Island and Socotra Rock in the Provisional Measure Zone between Korea and China. Water samples from four stations (St. 1, N 32°10′, E 125°05′; St. 2, N 32°35′, E 126°15′; St. 3, N 33°32′, E 126°10′; St. 4, N 33°32′, E 127°15′) were collected using a hand-operated Van Dorn-type sampler. In April, only surface layers were sampled. However, in September 2011, three different depths, i.e. the surface and 30- and 50-m depths, were sampled. Additionally, samples for environmental DNA extraction were prepared as follows: First, large size organisms such as zooplankton were removed using a 200 μm-sized mesh sieve. A total of 500 mL of the pre-filtered freshwater was size-fractionated sequentially through 10 μm (Cat. No. TCTP04700, 47 mm diameter, Millipore, Billerica, MA), 2 μm (TTTP04700, 47 mm diameter, Millipore), and 0.22 μm membrane filters (GVWP04700, 47 mm diameter, Millipore) to prevent clogging. The membrane filters were immersed in 0.8 mL extraction buffer (100 mM Tris–HCl, 100 mM Na_2-EDTA, 100 mM sodium phosphate, 1.5 M NaCl, 1% CTAB) and stored at −80 °C until DNA extraction.

2.2. Extraction of environmental DNA

DNA from the filtered samples was extracted using a modified form of the protocol employed by Harder et al. (2003). A 2-mL microcentrifuge tube containing each membrane filter (i.e., 10.0, 2.0, and 0.22 μm) was subjected to freeze-thaw cycles in liquid N_2 and a 65 °C water bath. Subsequently, 8 μL of proteinase K (10 mg mL^{-1} in TE buffer) was added and the tube was incubated at 37 °C for 30 min. Following incubation, 80 μL of 20% sodium dodecyl sulphate (SDS) prepared in double distilled water (ddH_20) was added, and the sample was incubated at 65 °C for 2 h. After incubation, the tubes were shaken with equal volumes of chloroform-isoamylalcohol (24:1) and centrifuged at 10,000 × *g* for 5 min. The aqueous phase of the mixture was transferred to a new microcentrifuge tube, to which 0.1 volume of 3 M sodium acetate (pH 5.1, prepared in ddH_20) and 0.6 volumes of isopropanol (≥99%) were added. The microcentrifuge tube was centrifuged at 14,000 × *g* for 20 min, the supernatant was discarded, 1 mL cold 70% ethanol was added to the pellet, and the sample was recentrifuged at 14,000 × *g* for 15 min. The pellet was air-dried and reconstituted in 100 μL TE buffer (10 mM Tris–HCl, 1 mM EDTA; pH 8) (Harder et al., 2003).