Biochemical Systematics and Ecology 70 (2017) 29–34

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Short communication

Molecular cloning reveals co-occurring species behind red tide blooms of the harmful dinoflagellate *Cochlodinium polykrikoides*

Ponmani Thangaraj [a], Tae Gyu Park [b], Jang-Seu Ki [a, *]

[a] Department of Life Science, College of Natural Sciences, Sangmyung University, Seoul 03016, South Korea
[b] Southeast Sea Fisheries Research Institute, National Institute of Fisheries Science (NIFS), Tongyeong 53085, South Korea

ARTICLE INFO

Article history:
Received 5 August 2016
Received in revised form 21 October 2016
Accepted 22 October 2016

Keywords:
Red tide
Harmful algal blooms
Cochlodinium
Polykrikos
Multiple dinoflagellates

ABSTRACT

Red tides caused by the marine dinoflagellate *Cochlodinium polykrikoides* Margalef pose significant environmental problems worldwide. Recently, the existence of severe blooms attributable to a single *Cochlodinium* Schütt species has been questioned by many researchers. Herein we investigated the dinoflagellate composition of harmful algal blooms (HABs) attributed to *C. polykrikoides* in Korean coastal waters at nine different stations (St.). The component species of *Cochlodinium* blooms were examined by using microscopic and gene-cloning methods. In the nine study areas, *C. polykrikoides* was the predominant species of HABs in St. 2, 4, 7, and St. 9. Based on the morphological identification, the bloom was initially thought to be caused only by *C. polykrikoides*; however, we detected additional bloom-forming dinoflagellates (*Polykrikos schwartzii* Bütschli and *Polykrikos kofoidii* Chatton), and diatoms (*Pseudo-nitzschia americana* (Hasle) Fryxell) along with *C. polykrikoides*. The parasitic dinoflagellates *Amoebophrya* Koeppen and *Euduboscquella* Coats, Bachvaroff & Delwiche were found to be co-located with *Cochlodinium* in our study, and for the first time, *Cochlodinium fulvescens* Iwataki, Kawami & Matsuoka was detected in Korea (west coast). These results suggest co-existence of multiple dinoflagellates in bloom populations of *Cochlodinium* and describe the composition of other dinoflagellate blooms (e.g., *Polykrikos* spp.) in Korean coastal regions. This co-occurrence may be considered during efforts to monitor and control HABs.

1. Introduction

Harmful algal blooms (HABs) are the consequence of the extreme growth of algae that adversely affect human health and aquatic ecosystems. Among the known microalgae species, 300 have been identified as HAB-forming, and approximately 40 species produce toxins that adversely affect marine animals and humans (Hallegraeff, 1993). Although historical evidence exists for the occurrence of HABs, their negative effects have aggravated dramatically over the last few decades, prompting global concerns over their increased frequency of occurrence (Guiry and Guiry, 2015). Likewise, HABs have drawn international socioeconomic interest due to their effects on ecosystems and human health. The Intergovernmental Oceanographic Commission (IOC) of the United Nations Educational, Scientific and Cultural Organization (UNESCO) formed an

* Corresponding author.
E-mail address: kijs@smu.ac.kr (J.-S. Ki).

http://dx.doi.org/10.1016/j.bse.2016.10.021
0305-1978/

Intergovernmental Panel on Harmful Algal Blooms (IPHAB) in 1987. IPHAB promotes active research on and effective management of HABs (see http://hab.ioc-unesco.org/).

Cochlodinium polykrikoides Margalef is an unarmored, major "red tide"-causing marine dinoflagellate. *C. polykrikoides* blooms have been reported in most parts of the world, including Southeast Asia, the east and west coasts of North America, and southwestern Asia and Europe; this global expansion of HABs has drawn the attention of researchers (Kudela and Gobler, 2012). *C. polykrikoides* produces ichthyotoxic substances (Richlen et al., 2010), including secreted mucopolysaccharides and reactive oxygen species (ROS), all of which damage fish gills (Kim et al., 2002). The toxicological basis of the *Cochlodinium* spp.-bloom ichthyotoxicity is not well understood although multiple factors appear to be involved (Kim et al., 2002). Thus, *Cochlodinium* blooms have the potential to cause major economic loss worldwide, particularly in Korea and Japan (Ahn et al., 2006; Anderson, 2009). Study of Lee et al. (2013) linked the regular and annual red tide events caused by *C. polykrikoides* in Korea since 1993. Cryptic speciation is reported in *C. polykrikoides*, in which four genetically different ribotypes were identified based on their sequences of 28S ribosomal RNA (rRNA). It includes the East Asian, Mediterranean, American/Malaysian, and Philippines ribotypes (Iwataki et al., 2008).

Park et al. (2013) reported genetically different *C. polykrikoides*, and co-location of other dinoflagellates such as *Alexandrium* Halim, *Akashiwo* Hansen & Moestrup, *Gyrodinium* Kofoid & Swezy, *Gymnodinium* Stein, and *Prorocentrum* Ehrenberg with *C. polykrikoides* in water columns in Korean coastal regions; together, these findings raise questions of whether *Cochlodinium* blooms are caused by a single-species or whether other species are involved. A morphological study by Rankin (2011) identified a *C. polykrikoides* bloom that included co-existing dinoflagellates *Prorocentrum micans* Ehrenberg, *P. gracile* Schütt and *Akashiwo sanguinea* (Hirasaka) Hansen & Moestrup in a California coastal region. However, few studies pertaining to this kind of research have been conducted (Rankin, 2011; Rinta-Kanto et al., 2005). Studies on the composition, bloom dynamics, and toxicological profile of algal blooms are imperative to the understanding of the nature of algal blooms and their effective management.

Hereby, we could detect the co-occurring species in *Cochlodinium* bloom in coastal area of Korea and this study might be helpful in identifying effective control methods to mitigate the impact of HABs. We extensively studied the dinoflagellate species composition of samples taken from *Cochlodinium* blooms using both microscopic/morphological analyses and molecular detection tools such as cloning and sequencing of DNA encoding for the 28S rRNA genes.

2. Materials and methods

2.1. Environmental sample collections

Surface water samples (3 m depth) were collected from nine different stations (St. 1–9) on the Korean coasts, including Gunsan, Tongyeong, and Yeongdeok (Fig. 1), from July to September 2013. The water samples were collected between 1.00 and 3.00 p.m. The range of water depth in the sampling stations is 15–35 m and they are located within 3 km from the shore. The temperature and salinity of the sampling areas were measured by using an Environment Monitoring System (YSI 6600, Yellow Springs Instruments, OH).

Water samples (100 mL) from the nine stations (Fig. 1) were preserved with Lugol's iodine solution (1–2% final concentration) and observed under a light microscope (Nikon eclipse 80i, Tokyo, Japan) at 100–1000 × magnification. The cell densities of dominant phytoplankton species were measured using a Sedgewick-Rafter counting chamber (Matsunami Glass Ind. Ltd., Osaka, Japan).

2.2. Environmental DNA extraxtion

The water samples (250 mL) were filtered using 1.2-μm pore-size, 47-mm diameter glass microfiber GF/C filters (Whatman, Ltd. Maidstone, England). Then the filter was transferred in to a 2-mL microcentrifuge tube, and stored at −80 °C until DNA extraction. DNA was extracted from the filtered samples following a protocol modified from that used by Harder et al. (2003). A 2 mL microcentrifuge tube containing the membrane filter was immersed in liquid nitrogen until completely frozen and thawed in a water bath maintained at 65 °C. Subsequently, 8 μL of proteinase K (10 mg mL^{-1} in TE buffer) was added to the microcentrifuge tube and 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, shaken with equal volume of chloroform–isoamylalcohol (24:1), and then 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 a 0.6 volume 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 centrifuged at 14,000 ×*g* for 15 min. The pellet was air-dried and reconstituted by adding 100 μL TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8).

2.3. PCR and molecular cloning

Polymerase chain reaction (PCR) was carried out from relevant dilutions of DNA with the following cyclic conditions: predenaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 20 s, 53 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 5 min. The primers used were 28F01 (forward) 5′ CCG CTG AAT TTA AGC ATA TAA GTA AGC 3′ and 28R691 5′ CTT GGT