J. Fish. Sci. Tech. 2(2), 172~175, 1999

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY
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Application of Image Analysis System for Red Tide Organisms

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(Received October 1999, Accepted December 1999)

Relative DNA contents in some harmful algae were measured using DAPI staining and image analysis system. This method was useful to identify some morphologically similar species and isolates from harmful algal blooms (HABs). In exponential phase, *Prorocentrum micans* had higher relative DNA content (RD) of 1.83 ± 0.52 than any other isolates, followed by *Cochlodinium polykrikoides* (1.10 ± 0.46), *Alexandrium tamarense* (0.93 ± 0.32), *Gyrodinium impudicum* (0.56 ± 0.17), *Scrippsiella trochoidea* (0.41 ± 0.26) and *P. minimum* (0.05 ± 0.01). When they were fixed with Lugol's solution, it was difficult to discern *C. polykrikoides* from *G. impudicum* under the light microscope, but the DNA contents were quite different in two species. *C. polykrikoides* contained about twice as much RD as *G. impudicum* under the same culture conditions and exponential phase. DAPI-stained DNA feature in *C. polykrikodes* showed concentrated in the peripheral part of the cell, but in *G. impudicum* showed a compact structure in the central part. Although *A. tamarense* and *S. trochoidea* were morphologically similar under the light microscope, nuclear DNA content of *A. tamarense* was twice as much as that of *S. trochoidea*.

Key words: Harmful microalgae, DAPI, image analysis, relative DNA area

Introduction

Several water-soluble DNA fluorochromes, such as ethidium bromide, acridine orange, Hoechst 332 58 (bisbenzimiditrihydrochloride, trihydrate), 4,-6,-diamidino-2-phenolindole (DAPI) and mithramycin are now commercially available to visualize the nuclei a organisms (Vrieling and Anderson, 1996; Goff and Coleman, 1990). DAPI and mithramycin give higher binding activity to nuclei a marine organisms than any other DNA fluorochromes (Goff and Coleman, 1990). It is known that mithramycin binds specifically to GC-rich regions of the genome, whereas DAPI binds to AT-rich regions. In view of quantum yield of DAPI and mithramycin-complexed DNAs, DAPI is more useful for detecting small amounts of DNA (Goff and Coleman, 1990). Accordingly, DAPI is currently used to identify and determine the distribution of bacteria (Enguchi and Kawai, 1992; Giorgio et al., 1996), and to assist nuclear DNA characterization at different developmental stages of macroalgae (Kapraun et al., 1996).

The amount of light emitted from a particular region of fluorochrome-stained DNA can be measured either by microspectrofluorometer or video interfaced digital image processor (Goff and Coleman, 1990). The microspectroflurometer enables us to measure total fluorescence flux emitted from a fluorochrome-stained region by using its detector, a photomultiplier tube (PMT). Thanks to this method, the number, size and position of nuclei in cells could be measured in macroalgae (Goff and Coleman, 1990). Some researchers analysed the nuclear DNA in *Gymnodinium mikimotoi* and *Chattonella* using microfluorometer system (Yamaguchi and Imai, 1994). Recently, Choi et al. (1994) applied the video interfaced digital image processor to the quantitative cytology in the life history of *Dasysiphonia chejuensis*. However, little studies were carried out on the nuclear state and DNA contents

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of microalgae in HABs from Korean coastal waters.

In the previous study, we had applied DAPI to harmful algae in order to observe and compare their nuclear features (Cho et al., 1999). In this study, we tried to determine the relative DNA contents of the HABs cells at the same culture condition and growth stage in the life history by employing the image analysis system.

Materials and Methods

Microalgae

Samples were taken from the red tide waters in Korea. Clones were established by isolating individual cell using capillary pipette under the light microscope. Six isolates were tested in this study (Table 1). They were grown in f/2-Si medium (Guillard and Ryther, 1962) containing an antibiotic mixture (Hasui et al., 1995). The culture room maintained constant temperature of 20℃, light intensity of 100 μmol m^{-2} S^{-1} and continuous light period with cool white fluorescent lamp. All algae are maintaining at the exponential growth phase in the Harmful Algal Research Division, National Fisheries Research and Development Institute.

Measurement of DNA content

Microalgal cells were treated with microwave for approximately 15 sec. and 1 μg/mℓ DAPI in Tris buffer 0.5 μg/mℓ (10 mM Tris, 10 mM EDTA-2Na, 100 mM NaCl, 10 mM 2-mercapto-ethylamine hydrochloride, pH 7.4) was added. After staining, treated cells were examined under epifluorescence microscope (Olympus BX50) attached with UV filter set (excitation, 330~385 nm, emission, >420 nm). For measurement of DNA content, the amount of light emitted from a particular region of fluorochrome-stained DNA was measured by image analysis system as described by Choi et al. (1994). Some microphotographs were recorded by scanner (Sharp, JX-330p, 24,000 DPI) and directly processed with personal IBM 586 computer. Quantification of DNA content in each cell was performed by use of image analysis software (Optimas 5.1 version for windows 3.1). This process was summarized in Fig. 1.

Table 1. Harmful algae used for this study

Strains	Isolation time	Location
Alexandrium tamarense	Feb. 1997	Chinhae
Cochlodinium polykrikoides	Sep. 1997	Tongyong
Gyrodinium impudicum	Aug. 1997	Tongyong
Prorocentrum micans	Oct. 1996	Masan
P. minimum	Aug. 1997	Tongyong
Scrippsiella trochoidea	Aug. 1997	Yosu

Results and Discussion

Nucleoid morphology

The morphology of nucleoids in 6 harmful algal annually occurring in Korean coastal waters was examined using fluorescence microscopy (Fig. 2). In *Prorocentrum micans*, the DNA-DAPI complex had a compact appearance without any distinct structures inside and were located in the central part of the cell, emitting intensive fluorescence (Fig. 2d). Fluorescence of DAPI-DNA complex in *P. minimum* showed a faint appearance of nucleoids compared with that in *P. micans* (Fig. 2f). The pattern of the DNA-DAPI complex of *P. minimum* was almost ovoid, whereas that of *P. micans* was elongated. Generally, it was painstaking to distinguish *Alexandrium tamarense* and *S. trochoidea* under the light microscope, but DNA-DAPI complex features in *A. tamarense* and *S. trochoidea* appeared significantly different. *Scrippsiella trochoidea* showed several bright spots of the fluorescence of DAPI-DNA complex (Fig. 2c), whereas *A. tamarense* showed a highly concentrated DAPI-DNA complex (Fig. 2a).

Partensky et al. (1988) suggested that two dinoflagellates, *Gymnodinium mikimotoi* and *Gyrodinium aureolum*, were morphologically indistinguishable under the light microscope, but it was possible to

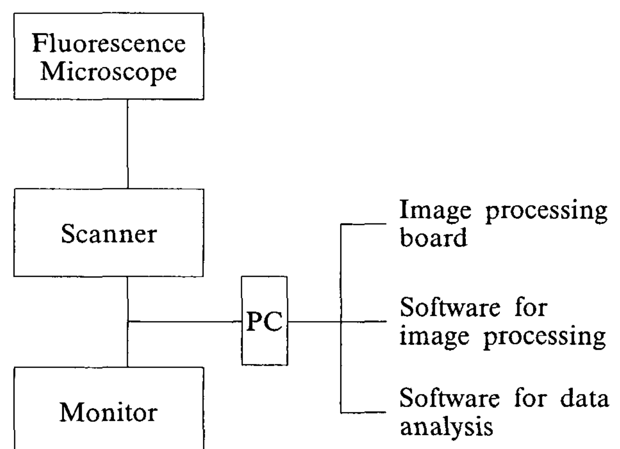

Fig. 1. Schematic process for DNA content using image analysis system.

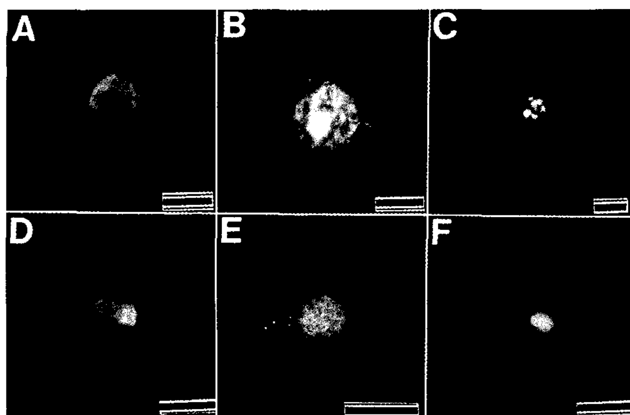

Fig. 2. Morphological features of DAPI-DNA complex (scale bar; A, B, C=20 μm, D, E=30 μm, F=10 μm). A: *Alexandrium tamarense*, B: *Cochlodinium polykrikoides*, C: *Scrippsiella trochoidea*, D: *Prorocentrum micans*, E: *Gyrodinium impudicum*, F: *Prorocentrum minimum*.

identify them easily from the morphology of DNA-DAPI complex. Likewise, DAPI may be used to discern between *A. tamarense* and *S. trochoidea*. Microalgae of *C. polykrikoides* and *G. impudicum* were also similar in morphology and difficult to identify under the light microscope. In *C. polykrikoides*, DAPI-DNA complex was seen to be concentrated in the peripheral part of the cell, interconnected by numerous thin filaments of DNA (Fig. 2 b), but *G. impudicum* was tightly condensed in the cell center, indicating that nucleoid represented compact structure (Fig. 2e). Although *C. polykrikoides* and *G. impudicum* were indistinguishable under the light microscope when they were fixed with Lugol's solution, it was possible to differentiate between them depending on the location of nuclei stained by DAPI. Therefore, DAPI staining may be used as an identification tool for some morphologically similar microalgae.

DNA content

As the image analysis system can easily quantify the relative DAPI-DNA contents it may be used as a quick HABs monitoring system. Choi et al., (1994) suggested that video interfaced digital image processor was able to measure DNA content stained by DAPI and to provide the quantitative cytology in life history of marine red algae. Fig. 3 shows the relative DNA content of DAPI stained nuclei of 6 isolates of red tide organisms under the same growth conditions and exponential phase. *P. micans* had the higher DNA content than any other isolate. *A. tamarense* and *C. polykrikoides* showed similar RD of 0.93 and 1.10, respectively. Also, *G. impudicum* and *S. trochoidea* showed a similar relative DNA content (RD) level of 0.56 and 0.41, respectively. *P. minimum* showed the lowest RD. Although *P. micans* and *P. minimum* belonged to the same genus and had similar nucleoid morphology stained by DAPI (Fig. 2d, f), DNA contents were significantly different ($p<0.05$).

C. polykrikoides and *G. impudicum* were similar in morphology under the light microscope, but their DNA contents were greatly different; C. polykrikoides showed much higher RD than G. impudicum during the exponential phase. In addition, *A. tamarense* and *S. trochoidea* could also be easily distinguished in relative DNA contents measured by the use of image analysis system (Fig. 3).

Conclusion

Using the scanning electron microscope, it takes several days to prepare the specimen to distinguish most of HABs. Moreover, it is not easy to get help from trained researchers and each day makes quite a difference in dealing with the HABs. It is very difficult to discern some toxic microalgae from its closely related non-toxic species under the light microscope. Therefore, it is necessary to develop quick and easy monitoring system.

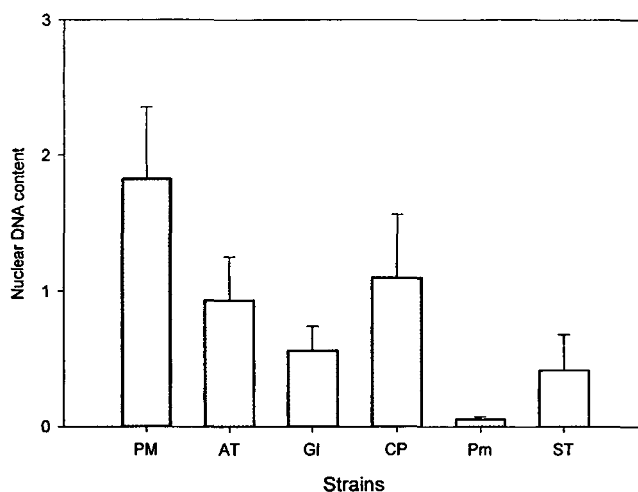

Fig. 3. Relative content of DNA in six red tide organisms analysed by image analysis system (PM: *Prorocentrum micans*, AT: *Alexandrium tamarense*, GI: *Gyrodinium impudicum*, CP: *Cochlodinium polykrikoides*, Pm: *Prorocentrum minimum*, ST: *Scrippsiella trochoidea*).

In this study, we have observed the structures of DNA-DAPI complex in six species of HABs. The results showed that we could easily distinguish morphologically similar species by the combined use of DAPI staining and image analysis system under the same growth phase and culture conditions. When combined with image analysis system, therefore, DAPI staining can be used as a very practical method to monitor HABs.

Acknowledgement

We thank Sung Ho Kim, Kobe University, for assistance and useful discussions. This study has been partially supported by internal grant of Kongju National University.

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