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論 文

Cell-meditated studies on blooming and growth of potentially ichthyotoxic *Cochlodinium polykrikoides* (Dinophyceae)

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ABSTRACT : The fluctuations of biochemical and molecular activities in the harmful dinoflagellate, Cochlodinium polykrikoides, depending on water temperatures, were studied. In genomic DNA concentration, a similar value of 0.6 was shown at 12℃ and 15℃, but significantly increasing DNA from 18℃ ($p<0.05$), with a maximum of 1.8 at 24℃. After 24℃, the DNA significantly decreased to 0.6. Likely, the concentrations of RNA and total protein were at their highest values of 1.7 and 0.07 g mL^{-1} at 24℃, respectively. In contrast to DNA, RNA and total protein began to increase at 15℃.Oxygen availability between lower and higher temperatures was significantly different and increased from 18℃ according to light intensity, regardless of wavelengths ($p<0.05$). At 24℃, the highest value of the maximum electron transport rate (ETRmax), ranging from 537.9 (Ch 1) to 602.5 mol electrons g^{-1} Chl a s^{-1} (Ch 4), was also shown. Nitrate reductase (NR) and ATPase activities were at their highest values of 0.11 mol NO_2^- g^{-1} Chl a h^{-1} and 0.78 pmol 100 mg^{-1} at 2 4℃, respectively. When the cells cultured at 15℃, NR and ATPase activities significantly increased compared to 12℃ ($p<0.05$). In an analysis of CHN, the concentration of C and N also significantly increased ($p<0.05$). However, at 27℃, most of the molecular and biochemical movements were much lower, compared to 24℃. These results suggest that C. polykrikoides is very sensitive biochemical and molecular activities depending on water temperatures. Possibly, it is desirable to estimate at 18℃ the initiation of the massive blooming development of C. polykrikoides. In nature, it will be very difficult to maintain the massive blooms after 24℃ because of the possibility of significantly decreasing the molecular movement and activity of C. polykrikoides.

KEY WORDS : biochemical activities, blooming, Cochlodinium polykrikoides, DNA, RNA, water temperature

1. Introduction

Most studies on bloom mechanisms and dynamics have concentrated on nutrient kinetics, assimilation, preferences, and uptake according to hydrographic properties. In contrast, little is known about growth characteristics of phytoplankton assemblages based on molecular studies, and enzyme activity. Relatively little attention has also been given to assessing the degree of biochemical and molecular processes of *C. polykrikoides* associated with the fluctuation of temperature. This study attempts to provide and analysis of the fluctuations of biochemical and molecular activities in *C. polykrikoides* in relation to main environmental factors inthe laboratory. Further, this study is an attempt to broaden my understanding of a cell biological magnitude to *Cochlodinium* blooms'process in nature.

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2. Material and Method

2.1 NR activity

Nitrate reductase (NR) activity was carried out as described in Joseph. Briefly, cells were filtered onto a 25 mm Whatman GF/C glass fiber filter and ground in extract buffer (200 mM phosphate buffer, pH 7.9, with 0.03% dithiothreitol (DTT), 0.3% polyvinyl pyrrolidone (PVP), 0.1% Triton X-100, 5 mM ethylene-diaminetetraacetic acid (EDTA), and 3% BSA. After grinding, homogenized suspension was centrifuged (300 × g, 4 min). The reaction was run in a final volume of 1.0 mL, containing 200 mM phosphate buffer, 0.2

mM NADH, 0.05 mM FAD, and 10 mM KNO_3. The time zero reaction was stopped immediately by the addition of 550 mM zinc acetate.

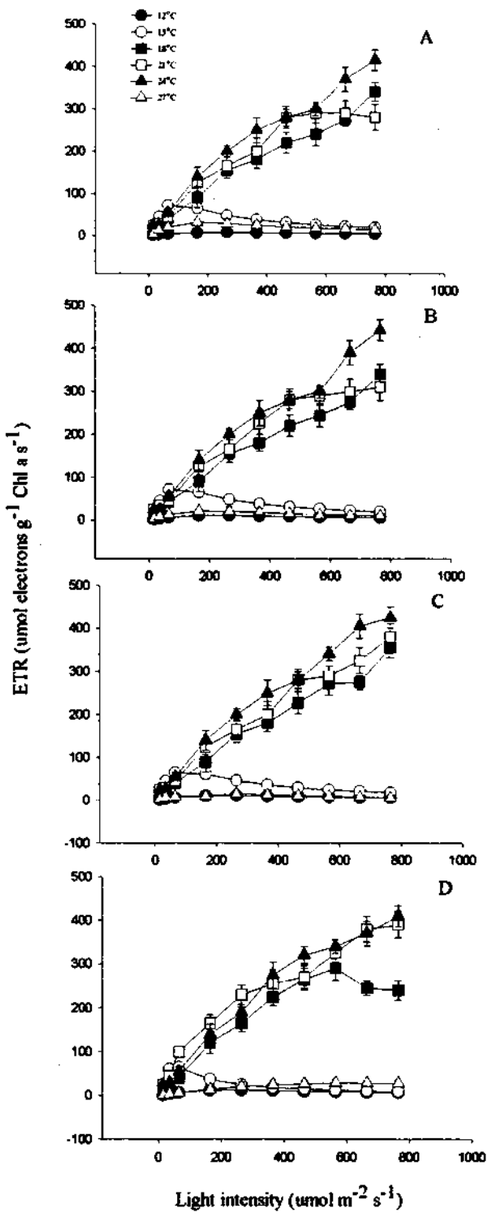

Fig. 1. Effect of *Cochlodinium polykrikoides* cultured at a different water temperature on ETR_{max} according to light intensity. The photosynthesis was measured by PHYTO-PAM with four wave lengths: Ch 1 (470 nm, A), Ch 2 (535 nm, B), Ch 3 (620 nm, C), and Ch 4 (650 nm, D). The error bar represents mean S.D. (n = 10).

3. Conclusion

The present study revealed that the highest concentrations and activities in *C. polykrikoides* based on biochemical and molecular analyses were 24℃, which was in agreement with previous studies. In nature, if the water temperature is at or near 24℃, massive blooms of *C. polykrikoides* will occur. In Japan, *C. polykrikoides* has different physiological features which are associated with a different water temperature for the occurrence of the blooms. However, Cho et al. reported that Korean strains of *C. polykrikoides* had identical nucleotide sequences. On the basis of molecular characteristics, massive blooms of *C. polykrikoides* will possibly occur in all Korean waters under 24℃ over a geographical barrier, but oceanographic conditions attempt to control the blooms. Interestingly, most cell-meditated movements remarkably decreased after 24℃ as shown in the present study. Possibly, it will be very difficult to maintain the massive blooms of *C. polykrikoides* in temperature higher than 24℃.

Reference

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