C-6

Reevaluation of the Generation of Reactive Oxygen Species (ROS) by *Cochlodinium polykrikoides* as a Fish Killing Factor; Comparison with *Chattonella marina*

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Introduction

Cochlodinium polykrikoides is one of the most harmful red tide dinoflagellates and is highly toxic to fish. Red tides due to this dinoflagellate have been reported in Korea, Japan, and other countries, and frequently cause severe damage to fish farming. Recently study has suggested that *C. polykrikoides* generates reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), and the ROS-mediated ichthyotoxicity has been proposed. 1) We and other groups have demonstrated that raphidophycean flagellates *Chattonella marina* and *Chattonella antiqua* generate ROS in a cell density dependent manner under the normal growth conditions. 2) On the other hand, no significant production of ROS was observed in other red tide phytoplankton species tested so far. Thus, this study was undertaken to reevaluate whether or not *C. polykrikoides* produces significant levels of ROS, and to gain the insight into the ichthyotoxic mechanism of this dinoflagellate especially its causative factors responsible for fish kill.

Methods

Cochlodinium polykrikoides was isolated in Yatsusiro sea, Japan and *Chattonella marina* was generously provided by Kagoshima Prefectural Fisheries Experimental Station in Japan. Generation of O_2^- by flagellates was measured by chemiluminescence method. Detection of H_2O_2 in the flagellate cell suspension was done by PHPA assay method. The cytotoxicities of the cell-free

extract prepared from algal cell suspensions against HeLa cells was measured by the inhibition of colony formation. Total polysaccharide levels in the culture supernatants of *C. polykrikoides* and *C. marina* were determined by the phenol-sulfuric acid method.

Result

We found that the levels of O_2^- and H_2O_2 detected in *C. polykrikoides* were trace levels as compared with those of *C. marina* which is well-known to produce ROS. Furthermore, no significant increase in O_2^- generation by *C. polykrikoides* was observed in the presence of lectins such as concanavalin A (Con A) and wheat germ agglutinin (WGA) or fish mucus prepared from skin and gill of yellowtail, whereas *C. marina* generated increased level of O_2^- responding to these stimuli. Interestingly, the cell-free aqueous extract prepared from *C. polykrikoides* showed toxic effect on the HeLa cells, but the extract of *C. marina* had no significant effect. Furthermore, gradual accumulation of polysaccharides in the medium was observed during the growth of a *C. polykrikoides*, and the medium gradually became viscous, but no such changes were observed in the medium of *C. marina*. These results suggest that *C. polykrikoides* may have the ichthyotoxic mechanism distinct from *C. marina*, and it is more likely that *C. polykrikoides* may exhibit fish toxicity through the secretion of biologically active metabolites such as toxin or mucus substances rather than through the generation of reactive oxygen species.

References

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