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High toxicity of the novel bloom-forming species *Chattonella ovata* (Raphidophyceae) to cultured fish

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Abstract

A toxicological study of an axenic cell line of novel species *Chattonella ovata* Y. Hara et Chihara (Raphidophyceae) revealed that cultured species of sea bream (*Pagrus major*), horse mackerel (*Trachurus japonicus*), and yellowtail (*Seriola quinqueradiata*) were killed by 4.1–6.8 × 10^3, 5.4 × 10^3, and 2.8 × 10^3 cells/mL, respectively. The sensitivity of the gill lamellae to *C. ovata* differed among the fish species tested. This finding revealed that *C. ovata* was highly toxic to the cultured fish. Histological examination showed that edema and hyperplasia of the secondary gill lamellae of red sea bream and horse mackerel occurred when exposed to, or killed by *C. ovata*, whereas severe damage in the gill lamellae was not observed in yellowtail. *Chattonella* produced high amounts of superoxide anion radicals and hydrogen peroxide, possibly responsible for the fish death observed. Based on the results of this study and occurrence of a red tide by this organism in China in 2001, we consider this organism to be one of the harmful algae in coastal waters. This is the first report demonstrating that *C. ovata* is highly toxic to fish, and that it produces superoxide and hydrogen peroxide.

Keywords: *Chattonella ovata*; *Pagrus major*; Red tide; *Seriola quinqueradiata*; Toxicity to fish; *Trachurus japonicus*

1. Introduction

The Raphidophyceae include several harmful red tide species, including *Chattonella antiqua* (Hada) Ono, *C. marina* (Subrahmanyan) Y. Hara et Chihara, *C. verruculosa* Y. Hara et Chihara, *Heterosigma akashiwo* (Hada) Hada, and *Fibrocapsa japonica* Toriumi et Takano. Of the seven species of the genus *Chattonella*, including *C. globosa* Y. Hara et Chihara, *C. minima* Y. Hara et Chihara, *C. ovata* Y. Hara et Chihara, and *C. subsalsa* Biecheler as described by Hara et al. (1994), the three species described above are known to be harmful. *C. antiqua* and *C. marina* are well known to cause mass mortality of cultured fishes in coastal areas around the world, and have been studied more than the other species.

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C. ovata has been reported to be a novel species of this genus, with a unique cell shape and sub-cellular organization (Hara et al., 1994). A bloom of this organism was recorded in Dapeng Bay in China on April 2001 (Songhui and Hodgkiss, 2001). This is the first record of a bloom caused by *C. ovata*, although it has often been observed in Harima-nada of the Seto Inland Sea and Kagoshima Bay in Japan. In 22 July 2004, the first known natural fish kill caused by this organism occurred. About 20,000 cultured yellowtail were killed in Kagawa, Japan. Here, we report that *C. ovata* was highly toxic to cultured fish in an experimental system.

2. Materials and methods

2.1. Cultivation of C. ovata

The axenic strain of *C. ovata* used in this experiment was isolated by I. Imai from Harima-nada (Japan) in 1993. It was cultivated in modified SWM-3 medium (Chen et al., 1969; Itoh and Imai, 1987) at 20 °C under 45 μmol/(m^2/s) illumination with a 12-h light:12-h dark photo-cycle.

2.2. Toxicological study

Pagrus major (65.1 ± 1.5 g), *Trachurus japonicus* (42.8 ± 1.7 g) and *Seriola quinqueradiata* (489.4 ± 66 g) were obtained from Fukui Prefectural Sea Farming Center, Hisatomi Suisan fishery company, and Fukui Prefectural Federation of Fisheries Co-operative Associations, respectively, and kept at Research Center for Marine Bioresources of Fukui Prefectural University. Eighteen liters of seawater was added to two polycarbonate tanks (30 L × 2), aerated with oxygen (5 mL/min) using a small oxygen disperser (Furuhashi Kiki, Japan). These tanks were put into a 1.0-m^3 water bath filled with water, and the temperature adjusted to 20 °C using a heat–cooling system (Miyahara Reinetsuki Kosakusho, Japan). Five red sea bream, five horse mackerel, or four yellowtail specimens were kept in smaller tanks for 1 day before the test. At the beginning of each experiment, 2 L of the *C. ovata* culture in a growing stage were added to one of the tanks (experimental tank), while the same volume of modified SWM-3 medium was added to the other tank (control tank). Initial cell densities of *C. ovata* are given in Tables 1 and 2. After the addition of *C. ovata* to the tanks, the fish were observed for 7 h. Dissolved oxygen (DO) concentration and pH of the seawater in the experimental and control tanks were measured using a DO meter (YSI Model 85, YSI, Japan) and pH meter (Twin pH B-212, Horiba, Japan). After the experiments, the gills of the fish were removed and fixed in 10% formalin for histological examination. Sections were stained with hematoxylin and eosin, or PAS. The ratio of histological alteration of the secondary gill lamellae of the fish was recorded as an alteration frequency ratios [(number of altered secondary gill lamellae/number observed) × 100].

Table 1
Toxicity of *C. ovata* to the fish *P. major*, *T. japonicus*, *S. quinqueradiata*

Experiment no.	Experimental fish	Cell density of *C. ovata* (×10^3 cells/mL)	No. of killed fish/No. of fish tested
1	*P. major*	0	0/5
		2.8	0/5
2	*P. major*	0	0/5
		4.1	4/5
3	*P. major*	0	0/5
		4.9	3/5
4	*P. major*	0	0/5
		5.1	5/5
5	*P. major*	0	0/5
		6.8	5/5
6	*T. japonicus*	0	0/5
		2.6	0/5
7	*T. japonicus*	0	0/5
		5.4	5/5
8	*S. quinqueradiata*	0	0/4
		2.8	4/4

2.3. Histological measurement of superoxide anion radicals and hydrogen peroxide produced by C. ovata

Superoxide anion radicals produced by *C. ovata* were measured by the cytochrome C method (Johnston et al., 1978). Hydrogen peroxide was measured by the method described by Nathan and Root (1977).

3. Results and discussion

C. ovata inoculated at a cell density of 10^2 cells/mL in modified SWM-3 medium proliferated to about