CULTURES OF DINOPHYSIS FORTII AND D. ACUMINATA WITH THE
CRYPTOMONAD, PLAGIOSELMIS SP.

T. ISHIMARU¹, H. INOUE², Y. FUKUYO², T. OGATA³ AND M. KODAMA³

¹Ocean Res. Inst., University of Tokyo, Nakano-ku, Tokyo (Japan)

²Faculty of Agr., University of Tokyo, Bunkyo-ku, Tokyo (Japan)

³School of Fisheries Sci. Kitasato Univ. Sanriku, Iwate (Japan)

Dinophysis fortii and D. acuminata, known as causative organisms of diarrhetic shellfish poisonings (ref. 1) have never been successfully cultured.

In our previous study (unpublished), D. fortii and D. acuminata survived for about a month showing at most two cell divisions under axenic conditions in enriched seawater medium. Addition of organic compounds such as yeast extract, sodium acetate, glucose, vitamins and hormones did not promote their growth. D. fortii survived for 5 months in filtered seawater (20- μ m mesh plankton net) containing bacteria and small flagellates. These results suggest phagotrophic nutrition of D. fortii.

In the present study, we cultured D. fortii and D. acuminata with various species of microalgae such as Synechococcus-type cyanobacteria, Isochrysis galbana, Pavlova lutheri, Nephroselmis sp., Dunaliella primolecta, Cryptomonas salina, Plagioselmis sp. and Symbiodinium microadriaticum. Behavior of Dinophysis was observed with a video camera attached to an inverted microscope. The *in vivo* fluorescence intensity of Dinophysis was measured by an electric exposuremeter during the culture (table 1).

When we cultured D. acuminata and D. fortii with the cryptomonad Plagioselmis sp., which occurred simultaneously with D. acuminata in Tokyo Bay, ingesting behavior by these Dinophysis were observed. The surface of Dinophysis cells appeared to become sticky after 2-3 hours from the beginning of dark period and Plagioselmis sp. were trapped to their surface. Then attached cells were covered with the cytoplasm of Dinophysis and absorbed inside cell within several hours. The following effects were observed when D. fortii was

cultured with Plagioselmis sp.: 1) decrease of the in vivo fluorescence was less significant (Table 1), 2) the frequency of the occurrence of defective cells was low (Table 1), 3) a single cell of D. fortii grew to make at most 22 cells in 3 weeks.

Our conclusion is that the ingestion of cryptomonads such as Plagioselmis sp. is necessary for the growth of D. fortii and D. acuminata. Chloroplasts ingested by these Dinophysis may remain intact and photosynthetically active for some time, such as diatom chloroplasts engulfed in Paulsenella chaetocoeratis (ref. 2). Orange or orange-red fluorescing bodies (ref. 3) or large chloroplasts observed in D. acuminata and D. fortii cells (ref. 4) may be the remaining chloroplasts of ingested algae.

Table 1. Decrease of in vivo fluorescence and the frequency of the occurrence of defective cells of D. fortii during the culture

		Rel. fluorescence*		defective cells
		max.-min.	mean+dev.	/observed cells
immediately after collection		200-120	154+24	----
after 2 weeks	in FSW [†]	77- 37	52+14	4/14
	in T/4 ^{††}	83- 37	59+12	7/17
	in T/4 with <u>Plagioselmis</u> sp.	100- 56	78+13	0/10

* Rel. fluorescence : 100/E, where E is an exposure time measured by electric exposure meter built in a photomicroscope system used with an epifluorescence microscope. Ten cells were measured in each observation. [†] Filtered seawater by GS millipore filter. ^{††} Enriched seawater medium (Ogata et al., 1987, Mar. Biol., 95, 217-220).

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