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Effect of water temperature and light intensity on growth rate and toxicity change in *Protogonyaulax tamarensis*

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Abstract

The effect of water temperature and light intensity on the growth rate and the toxicity of *Protogonyaulax tamarensis* was examined using a monoclonal culture isolated from Ofunato Bay, Japan in March, 1984. The growth rate decreased with the decrease of either light intensity or temperature. The amount of toxin produced increased concomitantly with the decrease of the growth rate. However, the increase of the toxicity under low growth rate was less remarkable when the growth rate was lowered by the decrease of light intensity. This indicates that photosynthesis plays an important role in the production of toxin in *P. tamarensis*.

Introduction

Protogonyaulax tamarensis is known to produce paralytic shellfish poison (PSP) (Prakash *et al.*, 1971; Singh *et al.*, 1982). Toxicity of this species has been suggested to be affected by some physiological or environmental factors, and thus it is different at various growth stages (Prakash, 1967; White and Maranda, 1978; Oshima and Yasumoto, 1979; Singh *et al.*, 1982; Boyer *et al.*, 1985). Salinity change affects the activity of the production of toxin in *Gonyaulax excavata* (*P. tamarensis*) (White, 1978). Production of toxin also changes during the course of the cell cycle (Kodama *et al.*, 1982). In a field survey, it was also suggested that the toxicity of *P. tamarensis* in an environment of lower temperatures is greater than that measured under higher temperatures (Ogata *et al.*, 1982).

Concerning the detailed relationship between the toxicity and growth rate of *Protogonyaulax tamarensis*, only a few findings have been reported. In this study, we examine the changes of toxicity in *P. tamarensis* under different conditions of light intensities and water temperatures.

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Materials and methods

Isolation and culture of the *Protogonyaulax tamarensis* clone

A single vegetative cell of *Protogonyaulax tamarensis* was isolated from Ofunato Bay, Iwate Prefecture, Japan in March, 1984, when this species was blooming. This clone (OF84423D-3) was mass cultured and maintained in enriched seawater T1 medium (Table 1) at 15 °C under an illumination of 3 000 lux with a 16 h light:8 h dark cycle, using cool-white fluorescent lamps. Culture experiments with different light intensities and water temperatures were carried out after acclimation of the clonal population to respective experimental conditions during a period of 20 d.

Measurements of growth rates

An aliquot of the cells was taken every day to count the cell density under a microscope. The growth rate (μ_2) was

Table 1. Enriched seawater T_1 medium

Compound [a]	Concentration (M)
Tris-HCl buffer (pH 8.0)	5×10^{-3}
$NaNO_3$	1×10^{-3}
NaH_2PO_4	1×10^{-4}
Fe-EDTA	5×10^{-6}
Thiamine HCl	5.93×10^{-7}
Biotin	4.1×10^{-9}
Cyanocobalamin	7.38×10^{-10}
$ZnSO_4$	1×10^{-6}
$MnCl_2$	1×10^{-5}
$NaMoO_4$	5×10^{-7}
$CoCl_2$	2×10^{-7}
$CuSO_4$	1×10^{-8}
EDTA (as Na_2 salt)	1.2×10^{-5}
Nitrilotriacetic acid (as Na_3 salt)	2×10^{-5}

[a] One ml of stock solution per liter filtered seawater gives the desired concentration

estimated from cell counts in the exponential phase using the following formula (Fukazawa *et al.*, 1980):

$$\mu_2 = \ln C_1 - \ln C_0/(T_1 - T_0) \ln 2,$$

where C_0 and C_1 indicate the cell number at times T_0 and T_1, respectively.

Toxicity and toxin composition of the cell

Protogonyaulax tamarensis is known to have different toxicities during the course of its growth stages (Prakash, 1967; White and Maranda, 1978; Oshima and Yasumoto, 1979; Singh *et al.*, 1982). Cell sizes of *P. tamarensis* also affect toxicity (Kodama *et al.*, 1982). Thus, each clonal culture was harvested at the end of the exponential phase using sieves with three different mesh openings as reported by Kodama *et al.* (1982). The 20- to 30-μm fraction, the most dominant fraction in every case, was sonicated with an equal volume of 0.2 N HCl for 2 min and then centrifuged at 3 000 rpm for 10 min. The extracts were boiled for 5 min to convert the N-carbamoyl derivatives of PSP into the corresponding strongly toxic components (Kobayashi and Shimizu, 1981). An aliquot, or its dilution, was injected into male mice (ddY strain, 18 to 22 g) to test toxicity. Toxicity was calculated from the time of death of the mice using the dose-death time table of PSP (Sommer and Meyer, 1937) and expressed in terms of mouse units (MU). One MU repesents a dose of PSP needed to kill a 20 g mouse within 15 min. The average cell volume of each fraction was calculated based on the cell number in the fraction and cell length and width of 100 cells selected at random. Toxicity of the cell was represented as MU per 10^4 cells and MU per mm^3 of cell quantity.

Toxin compositions of the extracts derived from the above procedure were analyzed by HPLC PSP analyzer (Oshima *et al.*, 1984). Based on the toxin composition, the molar concentration of PSP was calculated and expressed as nmol per 10^4 cells.

Results and discussion

Growth rates of *Protogonyaulax tamarensis* under different water temperatures and light intensities

Protogonyaulax tamarensis of the present clone were cultured under three different temperatures (8°, 12° and 16°C). Typical growth curves are illustrated in Fig. 1a. Growth rate decreased significantly with the decrease of temperature. When this species was grown under different light intensities (500, 1 000 and 3 000 lux), the growth rate at 15°C also decreased with the decrease of light intensity (Fig. 1b). In Fig. 2, growth rates are plotted against temperature (a) and light intensity (b) to indicate the results from different aspects. The growth rate decreased with the decrease of either temperature or light intensities under the experimental conditions tested.

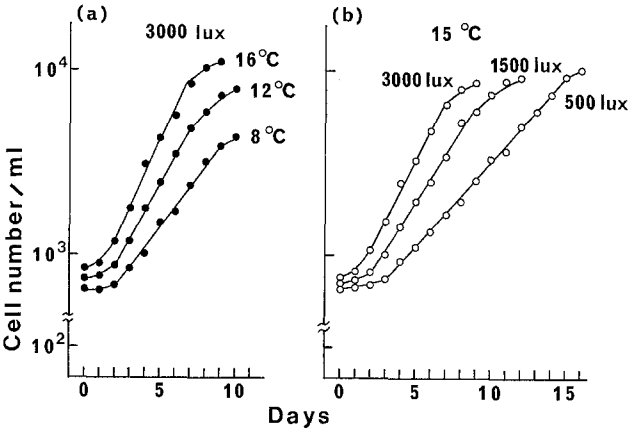

Fig. 1. *Protogonyaulax tamarensis.* (a) Growth curve at different temperatures (8°, 12° and 16°C) under a light intensity of 3 000 lux; (b) Growth curves obtained under different light intensities (500, 1 500 and 3 000 lux) at 15°C

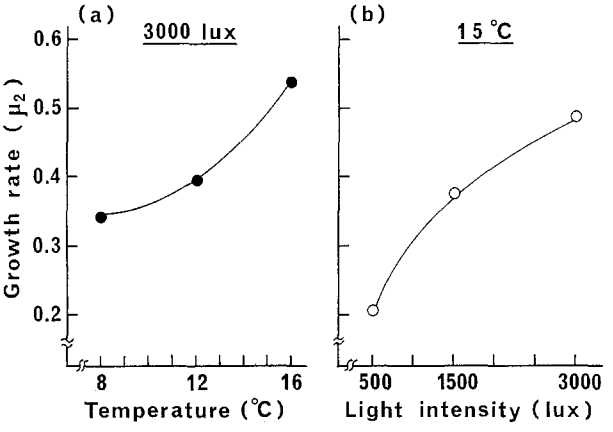

Fig. 2. *Protogonyaulax tamarensis.* (a) Growth rates under three different temperatures and (b) light intensities

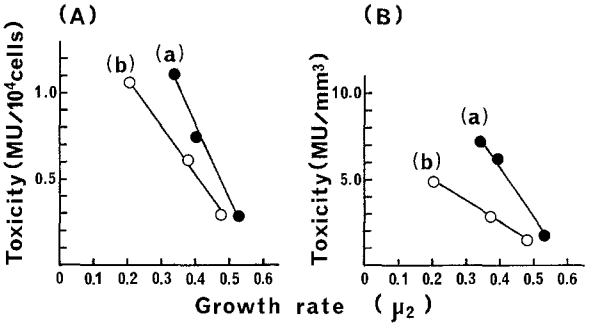

Fig. 3. *Protogonyaulax tamarensis.* Relation between toxicity and growth rates measured in different water temperature (a) and light intensity (b). Different toxicity formulas are used in A and B

Effect of growth rate on the toxicity of *Protogonyaulax tamarensis*

Fig. 3 shows the toxicity of *Protogonyaulax tamarensis* with respective growth rates, which were measured with different water temperatures and light intensities. Toxicity increased proportionally with the decrease of growth rate by lowering temperature (A–a in Fig. 3) or light intensity

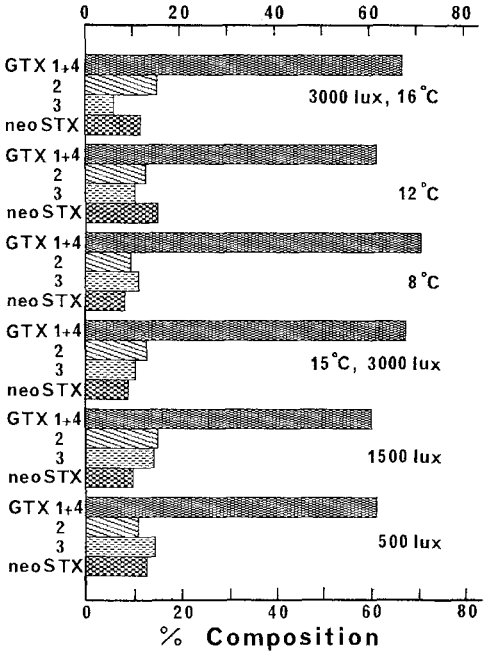

Fig. 4. *Protogonyaulax tamarensis.* Toxin composition under different temperature and light intensity conditions

Table 2. *Protogonyaulax tamarensis* (OF84423D3). Effect of temperature and light intesity on toxin concentration

Experimental conditions	Total toxin concentration[a] (nmol/10^4 cells)
3 000 lux, 16 °C	0.182
12 °C	0.470
8 °C	0.675
15 °C, 3 000 lux	0.201
1 500 lux	0.383
500 lux	0.657

[a] Calculated from the toxin composition shown in Fig. 5

Table 3. *Protogonyaulax tamarensis* (OF84423D3). Effect of slowdown of the growth rate on toxicity

Treatment	Harvesting	Toxicity		Total toxin concentration[a] (nmol/10^4 cells)
		MU/10^4 cells	MU/mm^3	
A[b]	Before treatment	0.68	2.30	0.491
	After treatment	1.63	4.84	1.007
B[c]	Before treatment	0.64	2.71	0.398
	After treatment	0.68	3.13	0.494

[a] Calculated from the toxin composition
[b] Treatment A: Growth rate was slowed by lowering temperature at the mid-exponential phase
[c] Treatment B: Growth rate was slowed by lowering light intensity at the mid-exponential phase

(A–b in Fig. 3), when it was expressed in MU per 10^4 cells.

Increase of toxicity per decrease of growth rate was higher when the growth rate was decreased by lowering the temperature than by lowering the light intensity. In the 20- to 30-μm fraction, there were cells of various sizes. Therefore, toxicity was represented as MU per mm^3 and plotted against growth rate. As shown in Fig. 3 B, the relation between toxicity and growth rate was similar to the results of Fig. 3 A.

Fig. 4 shows the toxin composition of *Protogonyaulax tamarensis* grown under different culture conditions. Although the toxicity changed in these conditions, the toxin composition did not change significantly. Table 2 indicates the molar concentration of toxin calculated on the basis of toxin composition. These data reveal that the increase in toxicity was not caused by conversion of the toxin components, but rather by the increase of the amount of toxin.

It has been pointed out that toxicity of *Protogonyaulax tamarensis* or *P. catenella* becomes higher when the growth rate in culture of these species is low (Proctor *et al.*, 1975; Singh *et al.*, 1982; Boyer *et al.*, 1985). In a field survey, we observed that the toxicity of *P. tamarensis* at low temperatures was higher than that obtained at higher temperatures (Ogata *et al.*, 1982). The results of the present study show that the increase of toxicity under a low rate of growth is less remarkable when the growth rate was influenced by light intensity rather than by temperature. This coincides well with phenomena observed in culture experiments (Proctor *et al.*, 1975; Singh *et al.*, 1982; Boyer *et al.*, 1985) and a field survey (Ogata *et al.*, 1982).

Effect of the slowdown of growth rate on toxicity of *Protogonyaulax tamarensis*

As described above, decreasing growth rate by lowering temperature and light intensity encouraged significantly the increase of toxicity of *Protogonyaulax tamarensis.* Therefore, the effect on toxicity by delaying growth rate during culture was tested. The temperature of the culture grown under 3 000 lux at 15 °C was lowered to 10 °C at the mid-exponential phase for a period of 4 d with identical illumination. Along with the temperature change, growth was slowed (Fig. 5 a). After this treatment, toxicity of the cells increased by up to more than twice that of before treatment (Table 3). A similar growth curve was also obtained in the present clone cultured under a light intensity of 3 000 lux at 15 °C, when the light intensity was lowered to 500 lux at the mid-exponential phase for a period of 4 d. Immediate delay of growth was also observed (Fig. 5 b), although the toxicity of the cell did not increase significantly (Table 3).

When sudden stress is applied, microorganisms generally stop cell division to adapt themselves to the new environmental conditions (Yanagita, 1981). The arrest of cell division observed in this study seems to be the result of such adaptation. Toxin was produced during the period of temperature decrease, but not produced with decreasing

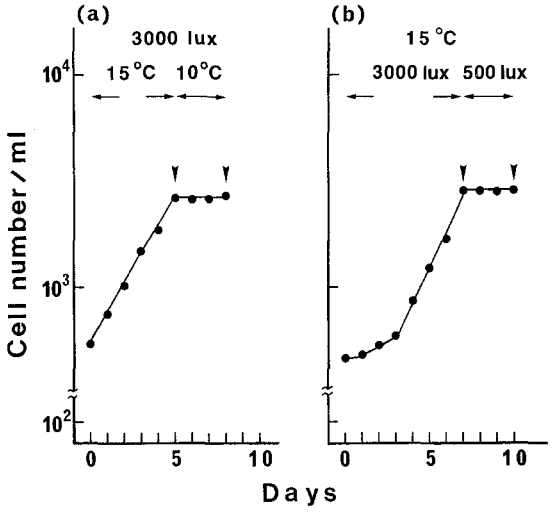

Fig. 5. *Protogonyaulax tamarensis.* Growth curves obtained by lowering the temperature (a) and light intensity (b) in the mid-exponential phase

light intensity. These results mean that ordinary photosynthesis is essential for the production of *Protogonyaulax tamarensis* toxin.

Few results have been reported on the biosynthesis of PSP in *Protogonyaulax tamarensis.* Shimizu *et al.* (1984) recently described saxitoxin analogs as being synthesized from some amino acids in this species. Glover *et al.* (1975) revealed that amino acids were produced during short periods of photoassimilation. We added NO_3 as a nitrogen source to the culture medium and therefore *de novo* synthesis of amino acids should have been carried out to utilize the incorporated NO_3. NO_3 assimilation of microalgae such as *Chlamydomonas reinhardtii, Chlorella pyrenoidosa, Ankistrodesmus braunii* and *Anabaena cylindrica* has been reported to be light dependent (Morris, 1974; Syrett, 1981), and this phenomenon has also been confirmed in *P. tamarensis* (MacIsaac *et al.*, 1979). Therefore, the decrease of light intensity in the mid-exponential phase of our experiments seems to reduce fresh synthesis of amino acids by suppressing nitrogen assimilation. The interruption of the production of toxin observed during the light retardation in the mid-exponential phase is seemingly dependent on suppressed *de novo* synthesis of amino acid due to the decrease of NO_3 assimilation. From these results, we conclude that *de novo* synthesis of amino acids by photoassimilation plays an important role in the production of toxin by *P. tamarensis.*

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