### Title
Studies on PSP Production in the Toxic Dinoflagellates Alexandrium catenella and Gymnodinium catenatum, and Intoxication Profile of the Short-necked Clam Fed with the Dinoflagellates

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CHAPTER III: Effect of light wavelength on the growth and PSP productivity of
*A. catenella* and *G. catenatum*

1. Introduction

In the previous chapter, the temperature seems to affect the growth and toxin production of toxic dinoflagellates that agreed with several published studies (Ogata et al., 1987; Anderson et al., 1990; Bravo et al., 1994). In the present chapter, the same strains of *Ac* and *Gc* were grown under different light wavelength (white, blue and red light) in order to investigate their effects on growth and PSP production.

As in natural environment, light under water is either absorbed or scattered in different direction. In facts, the absorbing particles are colored, absorption varies with wavelength causing certain colors of the visible spectrum to penetrate deeper into the water than others. The visible spectrum (white) ranges in wavelength from 400-700 nm. According to the colors, red and blue have wavelength ranging from 650-700 nm and 450-500 nm. In clear water, the level of penetration is generally greater for colors with shorter wavelengths and less for colors with longer wavelengths.

The purpose of our experiment was to determine if the light that toxic dinoflagellate absorbs could affect on the growth and toxin production. The red light would be absorbed the most by dinoflagellates at the surface, and blue light would be absorbed the most by dinoflagellates at the bottom since blue lights penetrates into the deepest. White light or visible spectrum was used as control for this experiment.
In this study, the first attempts were made to investigate the effects of light wavelengths on the PSP productivity of \textit{Ac} and \textit{Gc} in laboratory scales. The strain was cultured under three different lights with respective wavelength and the growth and PSP productivity were monitored.

2. Effects of light on the growth of \textit{Ac} and \textit{Gc}

2.1 Materials and methods

2.1.1. \textit{Ac} and \textit{Gc} cells

\textit{Ac} and \textit{Gc} samples were obtained and established from the same strain as described in CHAPTER I.

2.1.2. Culture conditions

Isolates of \textit{Ac} and \textit{Gc} were maintained in SWM-3 medium with similar conditions as explained in CHAPTER I, with slight modification of light intensity (60 \( \mu \)mol/m\(^2\)/s). The stock culture was preconditioned and incubated under three fluorescent lamps (white, blue and red), which represent different wavelengths (Fig. 16) in the same incubator within 21-30 days. The cells in logarithmic phase in respective preconditioning culture were inoculated in triplicate into flasks containing 500 ml of culture medium at about 200 cells/ml. They were grown with no stirring at each fluorescent light without changing the other conditions. The cell number was counted every two days during the culture period, and growth curves were drawn based on cell density and culture period for each fluorescent light.
Fig. 16. Spectral distribution of light wavelength for white, blue and red fluorescent lamps.
2.2. Results and discussion

The growth curves of \( Ac \) and \( Gc \) cells at each light wavelength of white, blue and red lights are given in Figs. 17 and 18.

The \( Ac \) cells showed the best growth in white and blue lights wavelength. The cells grew rapidly in white and blue light and reached the maximum density at 28 days of culture with 4,242 and 4,472 cells/ml, respectively. Meanwhile in red lights, cells grew slowly and attained maximum cell density, 1,346 cells/ml at 28 culture days and decreased thereafter.

\( Gc \) cells in all light colors were well grown and reached the stationary phase from 16 to 18 culture days. But, the cells grew rapidly in white and blue light and reached the maximum density, 2,558 and 2,442 cells/ml, respectively. In red lights incubator, the growth was fairly slower than in white and blue lights. However, the cell attained the maximum density 1,777 cells/ml at 16 days.

From the above results, the light wavelength seemed to give the effect on cell growth of both \( Ac \) and \( Gc \). The cells showed good growth rates in white and blue light, but much poorer growth under red light.
Fig. 17. Growth curves of *Ac* at three different light wavelengths.
Fig. 18. Growth curves of $Gc$ at three different light wavelengths.
3. Effect of different light wavelength on PSP productivity of Ac and Gc

3.1. Materials and methods

3.1.1. Samples
Approximately $10^6$ of Ac cells was harvested at 10, 20, 30 days in each light color. For Gc, the cell density about $10^5$ was used as samples for analysis that had been collected at 7, 14, 21, 28 days.

3.1.2. Toxin extraction
The cell pellet of each species Ac and Gc was separated from medium by centrifugation at 1500 g for 10 min. The pellet obtained was disrupted by mild sonication for three times (30 s each), in 0.5 M AcOH. After centrifugation at 3,000 g for 10 min, the supernatant was made up to 5 ml with distilled water. The prepared test solution from cells was ultrafiltered through an Ultrafree-PFL (Millipore) with cut-off limit of 10,000 daltons before subjected to HPLC-FLD analysis.

3.1.3. HPLC-FLD analysis
HPLC-FLD analyses was performed according to the procedures of Oshima, 1995b and Arakawa et al., 1995, as described in CHAPTER I. Toxin components were identified from the retention times of corresponding authentic ones.
3.2. Results and discussion

3.2.1. Toxin production of Ac and Gc cells

As shown in Fig. 19, the amount of Ac cell toxin (nmol/flask) was slightly varied among growth phases as the effect of light wavelength. Overall, the cells produced a larger amount of toxin under blue light (12.0) than under white light (6.7) especially in the exponential growth phase. Meanwhile, the cells that cultured in the red light gave lower toxin production (3.2–7.4) as compared with white (6.7–8.6) and blue (12.0–12.6) light during culture period.

The amount of toxin (nmol/flask) in the Gc cells was also different among growth phases under three different lights (Fig. 20). In early growth phases, cells grown in white light (40.6) recorded higher toxin amount compared with blue (25.5) and red (27.4) light. However, at the exponential growth phase, toxin amount in the blue light (119.1) was much higher than in red (79.9) and white (69.3) light. Towards the end of culture period (28th day), the amount of toxin varied remarkably; blue light (44.6) was much greater than white (29.1) and red (25.3) light. This might reflect the accidental decrease of cell density at that time as the cell became weaker towards the end of culture period.

As a summary, both Ac and Gc generally produced a larger amount of toxin under blue light than under white light that mostly observed in the exponential growth phase. Meanwhile, the red light usually gave lower toxin productivity than white and blue light during growth phase.
Fig. 19. Amount of Ac cell toxin cultured under white, blue and red lights.
Fig. 20. Amount of Ge cell toxin cultured under white, blue and red lights.
3.2.2. Toxin composition of Ac and Gc cells

Fig. 21 shows the PSP composition (mol%) of Ac cell toxin in each growth phase of white, blue and red lights. For each light, Ac cells produced C2, GTX1, 4 and 5 of four PSP components. C2 (2-15 mol%) and GTX4 (2-12 mol%) were the predominant toxins throughout the growth phases. In comparison of toxin composition with culture period (growth phase) there were differences among lights wavelength. White and blue lights, on 20th culture days, showed differences as the proportions of GTX1, 4 reduced as the cells grew. Meanwhile the mol% of GTX4 increased.

The HPLC results of PSP composition (mol%) of Gc cell toxin in each culture periods of different wavelength lights are described in Fig. 22. In general, the toxin composition of Gc grown under white and red light gave no change of toxin composition, while in blue light proportions of GTX5,6 were slightly increased. In white lights at 21 days, ratio of C1 to C2 was greatly changed. Transformation of toxin probably took place during extraction process.

From above results, it was obvious that there was no remarkable difference in toxin composition among the Ac or Gc cells grown under white, blue and red light.
Fig. 21. PSP composition (mol%) of Ac cells.
Fig. 22. PSP composition (mol%) of Gc cells.